JAN 27 1939

VOLUME 37 NUMBER 1

MEDICAL LIBRARY

JOURNAL OF BACTERIOLOGY

OFFICIAL ORGAN OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

JANUARY, 1939

EDITOR-IN-CHIEF
C.-E. A. WINSLOW

It is characteristic of Science and Progress that they continually open new fields to our vision.—PASTEUR

Published Monthly by THE WILLIAMS & WILKINS COMPANY
MT. ROYAL AND GUILFORD AVES., BALTIMORE, U. S. A.

Copyright, 1939, The Williams & Wilkins Company

Made in United States of America

Sustaining Members

Society of American Bacteriologists

American Instrument Co.	Silver Spring, Md.
American Sterilizer Company	Erie, Pa.
Anheuser-Busch, Inc.	St. Louis, Mo.
Bausch & Lomb Optical Company	Rochester, N. Y.
Braun-Knecht-Heimann Co.	San Francisco, Cal.
Coca-Cola Company	Atlanta, Ga.
Cutter Laboratories	Berkeley, Cal.
Difco Laboratories, Inc.	Detroit, Mich.
Eimer and Amend	New York, N. Y.
J. B. Ford Company	Wyandotte, Mich.
Gilliland Laboratories	Marietta, Pa.
Hynson, Westcott & Dunning	Baltimore, Md.
Kimble Glass Company	Vineland, N. J.
Lederle Laboratories	New York, N. Y.
E. Leitz, Inc.	New York, N. Y.
Eli Lilly & Company	Indianapolis, Ind.
Mead Johnson and Company	Evansville, Indiana
National Aniline & Chemical Company, Inc.	New York, N. Y.
Parke, Davis & Company	Detroit, Mich.
Pfaltz & Bauer, Inc.	New York, N. Y.
Redman Scientific Co.	San Francisco, Cal.
Scanlan-Morris Company	Madison, Wisc.
Sheffield Farms Company, Inc.	New York, N. Y.
Spencer Lens Company	Buffalo, N. Y.
Arthur H. Thomas Company	Philadelphia, Pa.
Will Corporation	Rochester, N. Y.
Wilmot Castle Company	Rochester, N. Y.
Carl Zeiss Inc.	New York City

Professional Directory Society of American Bacteriologists

THE Council of the Society has authorized the establishment of a directory where *those who desire to fill vacancies* may secure a selected list of applicants. This list is composed of teachers of bacteriology, technicians in laboratories, applicants for fellowships and scholarships in bacteriology serology, immunology, and research workers in medical or agricultural bacteriology. Employers may receive information regarding applicants without charge. Information regarding vacancies should be filed upon a blank which has been prepared to secure uniform information regarding a particular vacancy.

Apply for application blanks at the following address: F. M. Meader, M.D., *Detroit Department of Health, Detroit, Mich.*

Employment Agency Society of American Bacteriologists

THE Council of the Society has authorized the establishment of an employment agency. Bacteriologists and technicians may learn of positions by making application to the address below. Applicants for positions if not members of the Society will agree to send to the agency 5% of their salary for the first three months. If the applicant is a member of the Society he will agree to send only 3% of his salary for the first three months.

All funds accruing to the Society in excess of necessary and actual expenses, after being audited by the Society will be transferred to the treasurer of the Society for the improvement and enlargement of its Journal, support of its research fellowship, collection of cultures of bacteria, etc.

Send for application blanks to F. M. Meader, M.D., *Detroit Department of Health, Detroit, Mich.*

Entered as second-class matter April 17, 1916 at the postoffice at Baltimore, Maryland, under the Act of March 3, 1879.
Subscription price $5.00 per volume. Single numbers, $1.00.

COMPARATIVE RESISTANCE OF RECENTLY ISOLATED AND OLDER LABORATORY STRAINS OF E. TYPHOSA TO THE ACTION OF CHLORAMINE

PAUL KABLER, G. O. PIERCE AND G. S. MICHAELSEN

Division of Preventable Diseases and Division of Sanitation, Minnesota State Department of Health, Minneapolis, Minnesota

Received for publication April 27, 1938

Earlier experimental data have been reported which showed the resistances of several strains of *Eberthella typhosa* and of the coli-aerogenes group to chlorine and chloramine. It was found that some strains of *E. typhosa* remained viable as long as, and in some instances longer than, members of the coli-aerogenes group. Considerable variation in the resistance to chloramine was noted among the strains of *E. typhosa* and also among the members of the coli-aerogenes group. Since the old laboratory strains of *E. typhosa* were usually killed in a materially shorter time than were recently isolated strains, it seemed probable that prolonged residence on artificial media had reduced resistance to the action of chloramine. It was the aim of this investigation to test the validity of the above mentioned indication.

MATERIALS AND METHODS

The strains of *E. typhosa* used, with the exception of T5 and 706, were isolated in the laboratories of the Division of Preventable Diseases. The accompanying key indicates the year of isolation, source and age of the various strains.

The water used, in all the experiments except one, consisted of a mixture of treated[1] water from the distribution main leading out of one of the city's filtration plants and that from the tap at the laboratory of the Division of Sanitation. This mixture was

[1] Treatment at the filtration plant consisted of pre-ammoniation, prechlorination, coagulation, sedimentation, filtration, post-ammoniation, and post-chlorination.

Key to strains used in experiments to determine the killing power of chloramine for E. typhosa

YEAR ISOLATION	STRAIN NUMBER	ORGANISM ISOLATED		DURATION OF PATIENT'S CLINICAL CONDITION WHEN SPECIMEN WAS COLLECTED
		Date	From	
1933	1659	Sept. 18	Feces	Carrier—typhoid denied
1934	2230	Aug. 25	Feces	Carrier—typhoid 1897
	2349	Sept. 4	Feces	Carrier—typhoid 1928
	2365	Sept. 4	Feces	Carrier—typhoid denied
	2463	Sept. 14	Feces	Case—16 days
	M650	Sept. 15	Gallbladder	Carrier—typhoid denied
	M687	Oct. 16	Blood culture	Case—16 days
1935	1513	July 30	Feces	Carrier—typhoid in 1929
	1520	July 30	Feces	Case—28 days
	1560	July 30	Urine	Case—42 days
	1679	July 25	Feces	Case—44 days
	1727	July 26	Urine	Case—8 days
	2537	Sept. 6	Feces	Case—47 days
	S23	Sept. 9	Feces	Carrier—typhoid denied
	2623	Sept. 10	Feces	Case—27 days
	S59	Sept. 20	Feces	Case—85 days
	S83	Sept. 19	Feces	Case—36 days
	S129	Oct. 2	Feces	Carrier—typhoid denied
	3080	Oct. 5	Feces	Case—35 days
	3539	Oct. 26	Feces	Case—34 days
	3802	Nov. 12	Feces	Case—14 days
	M711	Nov. 18	Blood culture	Case—21 days
1936	2201	July 10	Feces	Carrier—typhoid denied
	2205	July 10	Feces	Carrier—typhoid denied
	2147	July 6	Feces	Carrier—typhoid denied
	2388	Aug. 3	Feces	Carrier—typhoid denied
	2587	Aug. 11	Feces	Case—8 days
	2697	Aug. 19	Feces	Carrier—typhoid 1907
	2792	Aug. 21	Feces	Carrier—typhoid denied
	2901	Aug. 31	Feces	Case—13 days
	S41	Sept. 1	Feces	Carrier—typhoid 1935
	S44	Sept. 1	Feces	Case—27 days
	S47	Sept. 1	Feces	Case—44 days
	S53	Sept. 4	Feces	Case—14 days

Old Lab. Strains: T5, in this Division since 1913; 706, Rawlings isolated in 1905.

used in order to obtain an initial chlorine residual between 0.20 and 0.23 p.p.m., the water from the tap having a much lower chlorine residual than that from the main. The water used

August 25, 1936, was drawn from the tap in the Division of Sanitation laboratory and used without mixing. The pH of the waters varied from 7.0 to 7.5, and the dissolved oxygen between 6.5 and 8.5 p.p.m. There was 0.2 p.p.m. or less of iron and 0.1 p.p.m. or less of manganese present.

The killing power of chloramine was determined for four strains of *E. typhosa* simultaneously, at two temperature ranges. The high range varied from 24.5 to 26°C., the low range from 1 to 3°C. The initial chlorine residual varied from 0.20 to 0.23 p.p.m.

For each day's experiment, 300 cc. of the water was placed in each of eight sterile 500 cc. Erlenmeyer flasks. Four of the flasks were subjected to the high, and four to the low range, temperature. After the water in the flasks had assumed the temperature of the bath, one flask at high temperature and one at low temperature were inoculated with[2] 0.6 cc. of a 1:10,000 dilution of a 24-hour broth culture of one of the strains to be studied. At fixed intervals one flask in each range was subsequently inoculated in a similar manner with a portion of a broth culture of the other strains to be studied. In the first four experiments, two 1 cc. portions were withdrawn from the flasks at 15 and 30 minutes, 1, 1½, 2, 2½ and 18 hours, and plated on brom-cresol-purple lactose agar. In the rest of the experiments the platings were made at the end of 10, 20, 30 minutes, 1, 1½, 2, 2½ hours. The plates were incubated 48 hours at 37°C., at which time the colonies were counted and recorded. The residual chlorine concentration was determined by the orthotolidine method at the beginning and at intervals throughout the course of the experiments.

RESULTS

The various plate counts were plotted on semi-logarithmic paper and the lines projected as described in a previous publication (Heathman *et al.*, 1936). It was found that, for approximately 60 per cent of the experiments, all of the plotted points were on, or very close to, the straight line. For about 30 per

[2] It had been found by previous experience that this amount of inoculum usually produced an initial bacterial population between 100 and 300 bacteria per cubic centimeter.

cent of the experiments, had the line been drawn through all the points, it would have produced a wavy line or one which was convexly curved in respect to the time axis, and for about 10 per cent a concavely curved line in the same respect.

Table 1 shows the time required to kill 99.9 per cent of the organisms as computed from the curves described. It will be seen that there are some variations in the killing time for any given strain. It may also be seen that the killing time required for the old laboratory strains on one day might exceed that for a freshly isolated strain on another day, but, usually, on any one day the time required to kill freshly isolated strains was greater than the time required to kill old laboratory strains. This was also true for the local strains which had been grown on artificial media one year or more. The average time required to kill T5 and 706 at high temperature was 0.767 hours, for the strains isolated in 1934, 1.239 hours, for those isolated in 1935, 0.901 hours, and for recently isolated strains 1.541 hours; for the low temperature it required 3.172, 4.821, 4.327 and 6.321 hours, respectively. It is also shown that there is considerable variation in the resistance of the strains within the various age groups. This is well illustrated by S59 and M711 compared to 2623 in the 1935 group and S44 and 2201 of the recently isolated strains.

Table 1 also shows the times required by chloramine of approximately the same chlorine residual to kill 12 strains of *E. typhosa* in 1935 as compared to 1936. It will be readily seen that the average time required to kill the old laboratory strains (T5 and 706) did not materially change from 1935 to 1936. There is some doubt as to any loss of resistance by strains 1679, 1727, and 2623 which were isolated in 1935. However, the resistances of 2537, M711, 3539, S23, 3080, S129, and S83 were markedly reduced. This is true at both the high and low temperature ranges.

The average time required to kill the strains of any group was from three to five times longer at low temperature than at high temperature.

The results obtained did not show any correlation between the time required to kill the organisms and the pH, dissolved oxygen, iron or manganese content of the water.

TABLE 1

Results of the action of chloramine on 36 strains of E. typhosa in 1936 including results for 12 of the same strains in 1935

E. TYPHOSA		DATE OF EXPERIMENT	INITIAL CL. RESID. OF WATER SAMPLE	HIGH TEMPERATURE			LOW TEMPERATURE			
Strain number	Year isolated			Degrees C.	Computed hours to kill 99.9%	Observed hours to kill†	Degrees C.	Computed hours to kill 99.9%	Observed hours to kill†	
			p.p.m.							
T5	‡	10/ 8/35	0.23	19–24	0.813	1	2–7	2.24	1½	
		10/17/35	0.23	17–27.5	1.74	1	2.5–6	4.18	2	
		8/19/36	0.22	25–26	0.60	½	2–3	2.15	2	
		9/ 9/36	0.22	24.5–25	1.9	1	1	4.85	>2½	
		8/24/36	0.22	25.5–26	*	*	2.5–3.5	2.05	1½	
		9/11/36	0.22	26	0.993	1	1.5–2	4.55	>2½	
		8/31/36	0.20	25	0.90	1	2	3.70	>2½	
		9/16/36	0.20	25–26	1.195	1	1–1.5	4.70	>2½	
706	1905	8/14/35	0.22	29–31	0.54	½	*	*		
		8/20/36	0.20	25.5–26	0.60	½	1.5–2.5	1.85	2	
		9/10/36	0.20	25	0.722	½	1	3.50	2½	
		8/25/36	0.22	24.5–25	0.60	½	2.5–3	2.1	2½	
		9/14/36	0.22	25	1.045	½	1–2	4.50	2½	
		9/ 1/36	0.22	25	0.60	½	1.5–2	1.55	1½	
		9/17/36	0.22	25–25.5	0.988	½	1–1.5	2.56	2	
1659	1933	8/19/36	0.22	25–26	0.600	½	2–3	2.0	2	
		9/ 9/36	0.22	24.5–24	1.49	1	1	7.50	>2½	
M650	1934	8/20/36	0.20	25.5–26	2.0	1½	1.5–2.5	6.3	>2½	<18
		9/10/36	0.20	25	3.15	1	1	6.15	>2½	
2230	1934	8/24/36	0.22	25.5–26	1.30	1	2.5–3.5	4.30	2½	
		9/11/36	0.22	26	1.30	1	1.5–2	7.65	>2½	
2349	1934	8/25/36	0.22	24.5–25	1.0	1	2.5–3	6.20	>2½	<18
		9/14/36	0.22	25	0.943	1	1–2	5.80	>2½	
2365	1934	8/27/36	0.23	25–26	0.85	½	2–3	4.20	>2½	
		9/15/36	0.22	24.5–25	1.238	1	1–2	5.25	>2½	

* No test.

† The numbers in these columns represent the elapsed time in hours from the initial inoculation of the flasks to the time when the first set of plates were made which contained no colonies of *E. typhosa*.

‡ In these laboratories since 1913.

TABLE 1—*Continued*

E. TYPHOSA		DATE OF EXPERIMENT	INITIAL Cl. RESID. OF WATER SAMPLE	HIGH TEMPERATURE			LOW TEMPERATURE		
Strain number	Year isolated			Degrees C.	Computed hours to kill 99.9%	Observed hours to kill†	Degrees C.	Computed hours to kill 99.9%	Observed hours to kill†
			p.p.m.						
2463	1934	8/31/36	0.20	25	0.795	1	2	3.62	>2½
		9/16/36	0.20	25–26	0.857	1	1–1.5	3.60	>2½
M687	1934	9/ 1/36	0.22	25	0.682	½	1.5–2	2.20	2½
		9/17/36	0.22	25–25.5	0.750	1	1–1.5	2.58	>2½
3802	1935	8/19/36	0.22	25–26	0.638	½	2–3	2.08	1½
		9/ 9/36	0.22	24.5–25	1.70	1	1	9.60	>2½
1560	1935	8/25/36	0.22	24.5–25	0.663	1	2.5–3	8.2	>2½
		9/14/36	0.22	25	1.80	1	1–2	15.0	>2½
1513	1935	9/ 2/36	0.22	25	0.60	½	2	2.60	2½
		9/21/36	0.22	25.5–26	0.448	½	1.5–2	2.1	1½
1520	1935	9/ 8/36	0.21	25.5	0.638	1	1.5–2.5	2.90	2½
		9/23/36	0.22	25	0.638	½	0.5–1	3.70	>2½
859	1935	9/ 8/36	0.21	25.5	0.575	½	1.5–2.5	2.50	2
		9/23/36	0.22	25	0.448	½	0.5–1	2.80	2
1679	1935	8/22/35	0.21	26–27	1.46	1½	•	•	•
		8/31/36	0.20	25	0.865	1	2	5.0	>2½
		9/16/36	0.20	25–26	1.55	1	1–1.5	8.75	>2½
1727	1935	9/ 4/35	0.23	22–23	2.32	1	•	•	•
		8/27/36	0.23	25–26	0.697	½	2–3	4.92	2½
		9/15/36	0.22	24.5–25	2.25	1	1–2	8.45	>2½
2537	1935	9/18/35	0.20	24–28	1.59	1	5–10	5.60	2
		10/ 1/35	0.20	22–24.5	0.813	1½	2–6	2.85	>2½
		9/ 1/36	0.22	25	0.682	½	1.5–2	2.58	>2½
		9/17/36	0.22	25–25.5	0.663	1	1–1.5	2.22	2
2623	1935	9/30/35	0.20	23–24	2.11	1	4–9	6.76	>2½
		8/27/36	0.23	25–26	0.825	1	2–3	4.40	>2½
		9/15/36	0.22	24.5–25	3.36	1	1–2	10.45	>2½

TABLE 1—*Continued*

E. TYPHOSA		DATE OF EXPERIMENT	INITIAL CL. RESID. OF WATER SAMPLE	HIGH TEMPERATURE			LOW TEMPERATURE		
Strain number	Year isolated			Degrees C.	Computed hours to kill 99.9%	Observed hours to kill†	Degrees C.	Computed hours to kill 99.9%	Observed hours to kill†
			p.p.m.						
3080	1935	10/ 7/35	0.23	22–26	3.73	1	2–8	7.20	>2½ <18
		10/16/35	0.23	22.5–24	2.18	1½	2.5–3	5.28	>2½ <18
		8/20/36	0.20	25.5–26	0.638	½	1.5–2.5	3.6	>2½ <18
		9/10/36	0.20	25	1.02	1	1	4.20	>2½
3539	1935	12/16/35	0.20	21–22	2.69	>1	3–4	4.62	>2½ <18
		9/ 2/36	0.22	25	0.793	1	2	3.17	2
		9/21/36	0.22	25.5–26	0.75	½	1.5–2	3.70	2
M711	1935	12/18/35	0.20	22	2.62	>1	3–4	*	*
		9/ 3/36	0.22	25	0.589	½	1.5–2	2.40	2
		9/22/36	0.22	25–26	0.339	½	2	0.92	1
S23	1935	10/ 2/35	0.22	23.5–27	1.50	1	3–7	4.62	>2½ <18
		8/24/36	0.22	25.5–26	0.60	½	2.5–3.5	1.07	1½
		9/11/36	0.22	26	0.556	½	1.5–2	1.80	1½
S83	1935	10/14/35	0.23	20–25	2.48	2	3.5–4	3.76	>2½ <18
		9/ 2/36	0.22	25	0.875	½	2	3.45	>2½
		9/21/36	0.22	25.5–26	0.71	½	1.5–2	2.83	2
S129	1935	10/ 9/35	0.23	20–25	1.87	1	1–8	*	*
		10/15/35	0.23	19–24	2.78	1½	2–9	6.28	>2½ <18
		9/ 3/36	0.22	25	0.732	½	1.5–2	3.38	>2½
		9/22/36	0.22	25–26	0.493	½	2	1.03	1
2147	1936	8/27/36	0.23	25–26	1.0	1	2–3	3.90	>2½
		9/15/36	0.22	24.5–25	1.34	1	1–2	6.50	>2½
2201	1936	9/ 1/36	0.22	25	2.07	1	1.5–2	6.75	>2½
		9/17/36	0.22	25–25.5	3.20	1	1–1.5	4.95	>2½
2205	1936	8/31/36	0.20	25	1.02	1	2	6.30	>2½
		9/16/36	0.20	25–26	1.388	1	1–1.5	5.26	>2½
2388	1936	8/20/36	0.20	25.5–26	1.7	1	1.5–2.5	5.20	>2½ <18
		9/10/36	0.20	25	3.25	1	1	11.70	>2½

TABLE 1—*Concluded*

E. TYPHOSA		DATE OF EXPERIMENT	INITIAL CL. RESID. OF WATER SAMPLE	HIGH TEMPERATURE			LOW TEMPERATURE		
Strain No.	Year isolated			Degree C.	Computed hours to kill 99.9%	Observed hours to kill†	Degrees C.	Computed hours to kill 99.9%	Observed hours to kill†
			p.p.m.						
2587	1936	8/19/36	0.22	25–26	0.60	½	2–3	1.6	2
		9/ 9/36	0.22	24.5–25	2.08	1	1	8.45	>2½
2697	1936	8/24/36	0.22	25.5–26	0.92	1	2.5–3.5	3.23	2½
		9/11/36	0.22	26	2.60	1	1.5–2	14.40	>2½
2792	1936	8/25/36	0.22	24.5–25	0.95	1	2.5–3	8.85	>2½
		9/14/36	0.22	25	2.90	1	1–2	13.10	>2½
2901	1936	9/ 2/36	0.22	25	1.06	1	2	5.0	>2½
		9/21/36	0.22	25.5–26	1.16	1	1.5–2	3.40	2½
S41	1936	9/ 3/36	0.22	25	1.17	1	1.5–2	6.80	>2½
		9/22/36	0.22	26	0.75	½	2	1.33	1½
S44	1936	9/ 3/36	0.22	25	0.837	½	1.5–2	5.20	>2½
		9/22/36	0.22	26	0.448	½	2	1.02	1
S47	1936	9/ 8/36	0.21	25.5	1.495	1	1.5–2.5	8.14	>2½
		9/23/36	0.22	25	1.195	½	0.5–1	4.18	>2½
S53	1936	9/ 8/36	0.21	25.5	2.60	1	1.5–2.5	11.35	>2½
		9/23/36	0.22	25	1.25	1	0.5–1	5.20	>2½

DISCUSSION AND SUMMARY

The waters used had an initial chlorine residual within the range of from 0.20 to 0.23 p.p.m. The final chlorine residual varied from 0.17 to 0.22 p.p.m., so that the average residual during the test in every instance approached 0.20 p.p.m. The temperature ranges were maintained within limits of 2° variation. With these variables reduced to a minimum, the foregoing results indicate that freshly isolated strains of *Eberthella typhosa* exhibit an appreciably greater resistance to chloramine than do strains that have resided on artificial media for an indefinite period of time. Strains of *Eberthella typhosa* isolated

in 1935 were killed in an average time of 2.18 hours when studied shortly after isolation. When they were retested in 1936, the average time required to kill them by chloramine of the same residual range and at the same temperature was 0.901 hours. Since the average time required to kill the old laboratory strains under the same conditions was 0.767 hours, it appears that, in one year, the strains isolated in 1935 lost a large part of the total probable decrease of resistance. This evidence points out a possible danger in the use of old laboratory strains in determinations of the effectiveness of chloramine as a disinfectant. It is again demonstrated that members of the freshly isolated group, as well as those that have been maintained on artificial media, exhibit considerable variations in their resistance to the action of chloramine. The possibility of error is apparent if conclusions are drawn from such determinations as those above mentioned if only one strain be used. It is also clearly shown that the killing time is lengthened from three to five times by lowering the temperature from a range of 24.5° to 26°C. to one from 1° to 3°C.

CONCLUSIONS

1. The resistance of *Eberthella typhosa* to the killing action of chloramine is materially reduced by prolonged residence on the usual artificial media.

2. Strains of *Eberthella typhosa* isolated at approximately the same time may exhibit considerable differences in resistance to the action of chloramine.

REFERENCE

HEATHMAN, L. S., ET AL.: Public Health Reports. 1936 **51**, 1367; Part III, Report on Investigation of Typhoid Fever Epidemic, Minneapolis, 1935.

NITROGEN METABOLISM OF CERTAIN COLIFORM BACTERIA

PHILIP L. CARPENTER

University of Wisconsin[1]

Received for publication May 4, 1938

Knight (1936) has stated that the nutritional requirements of the coliform bacteria are apparently quite simple. As reported by Koser and Rettger (1919), all strains of *Escherichia coli*, *Escherichia acidi-lactici*, *Aerobacter aerogenes*, and *Aerobacter cloacae* tested were able to grow in continued subculture in a medium of salts with ammonia or a single amino acid as source of nitrogen, glucose or glycerol being supplied as carbon source. Amino acids which were suitable nitrogen sources for these organisms were glycine, lysine, histidine, aspartic acid, tryptophane and tyrosine. Braun and Cahn-Bronner (1921) grew *E. coli* aerobically with ammonium lactate as nitrogen-carbon-energy source.

Koser (1918) reported that *A. aerogenes* was able to grow well in a synthetic medium containing uric acid as the sole source of nitrogen, whereas *E. coli* was unable to do so. He suggested that the differentiation was due to the inability of *Escherichia* strains to break down the purine ring and thus obtain nitrogen for growth from the uric acid. Several other investigators have studied utilization of purine derivatives by coliform bacteria. Mitchell (1937), in a comprehensive study of 360 strains of *Escherichia*, *Aerobacter* and *Citrobacter*, pointed out some interesting relationships. She determined the ability of these bacteria to grow in synthetic media containing various purine derivatives as sole source of nitrogen. *Aerobacter* strains grew well in all media tested: yeast nucleic acid, uric acid, allantoin, hydantoin,

[1] Present address: Dept. of Bact., Iowa State College, Ames, Iowa.

uracil, urea, adenine sulphate, and xanthine. *Escherichia* strains grew only in uracil, adenine sulphate, and xanthine; while the cultures of *Citrobacter* grew only in urea, adenine sulphate, and xanthine.

Bacteria of the genus *Aerobacter* are commonly divided into two groups on the basis of ability to liquefy gelatin. Those which liquefy gelatin include *A. cloacae*. Although these bacteria are able to destroy the solidifying power of the protein, they are unable to utilize the liquefied substance, as was shown by Berman and Rettger (1918a). These authors also found that this organism will not attack proteoses or coagulated proteins.

One of the first quantitative studies of protein metabolism was that of Kendall and Bly (1922). These investigators made periodic analyses of 0.5 per cent gelatin-broth cultures of *E. coli* to follow changes in soluble, amino and ammonia nitrogen, and by calculation obtained values for "polypeptide" (non-amino, non-ammonia, soluble) nitrogen. Their data show that the chief change in the nitrogenous constituents of the culture medium occurred in the "polypeptide" fraction, and that there was little change in amino nitrogen. Ammonia was produced after several days, due possibly to deamination of amino acids by the organisms in search for energy. This was considered probable in view of the fact that no ammonia was liberated in glucose broth cultures. The authors concluded that no extracellular proteolytic enzyme was produced by *E. coli*.

There has been relatively little quantitative work done on nitrogen metabolism of any coliform bacteria except *E. coli*. It was therefore considered desirable to study the utilization of various forms of nitrogen by representative strains of *E. coli*, *A. aerogenes*, and an *Escherichia-Aerobacter* intermediate.

METHODS

The bacteria employed in this investigation were typical strains of *E. coli* and *A. aerogenes* obtained from Dr. B. W. Hammer, and a citrate-positive, methyl-red-positive, Voges-

Proskauer-negative *Escherichia-Aerobacter* intermediate obtained from Dr. C. H. Werkman.

The experimental procedure consisted of periodic analysis of cultures in media containing ammonium chloride or peptone as source of nitrogen, in addition to glucose and dibasic potassium phosphate. Quantitative determinations were made for total nitrogen, non-protein or tungstic acid-soluble nitrogen, ammonia nitrogen, and alpha-amino nitrogen. Soluble nitrogen was determined by the micro-Kjeldahl method on tungstic acid filtrates of the cultures, absorbing the liberated ammonia in 5 per cent boric acid and titrating with standard N/100 acid. Ammonia nitrogen was determined by the Folin aeration method employing solid sodium carbonate to liberate the ammonia, which was absorbed in N/100 acid; excess acid was titrated with standard N/100 NaOH. Alpha-amino nitrogen was determined by the Van Slyke method using a micro-apparatus on the residue left after determination of ammonia. All procedures were checked for recovery of added nitrogen and standardized to give uniform and accurate results.

The composition of the media employed is indicated in the tables of results. Media were dispensed in 25 ml. amounts in six-ounce bottles. These were inoculated with one drop of 24-hour cultures of the bacteria growing in the same medium. Incubation was at 35–37°C. For each analysis triplicate bottles of the same culture were pooled, and duplicate determinations made on the pooled material.

UTILIZATION OF AMMONIA

Utilization of ammonia nitrogen by coliform bacteria was determined by analysis of three-day cultures in a medium containing 0.08 per cent NH_4Cl as source of nitrogen. The results are shown in table 1. The decrease in ammonia nitrogen ranged from 12.8 per cent in the case of *E. coli* to 21.5 per cent in the *A. aerogenes* cultures. The figures show that all three coliform strains possess the ability to derive their nitrogen from an ammonium salt, but that there are no striking differences between the strains with respect to the amounts required.

UTILIZATION OF PEPTONE NITROGEN

Cultures containing peptone as source of nitrogen and glucose as source of carbon and energy were analyzed after two, four,

TABLE 1

Utilization of ammonia nitrogen by coliform bacteria

Medium: NH_4Cl (0.08 per cent), glucose (0.5 per cent), K_2HPO_4 (0.5 per cent); pH 7. Age: 3 days.

CULTURE	NH_3-N IN MGM. PER 100 ML.
Control	21.8
E. coli	19.0
A. aerogenes	17.1
Intermediate	17.7

TABLE 2

Utilization of peptone nitrogen by coliform bacteria

Medium: peptone (0.5 per cent), glucose (0.5 per cent), K_2HPO_4 (0.5 per cent); pH 7. Nitrogen in milligrams per 100 ml.

CULTURE	AGE	TOTAL N	PROTEIN N	NON-PROTEIN N	DIFFERENCE N	AMINO N	AMMONIA N
	days						
E. coli	0	88.6	62.6	26.0	9.6	16.3	0.1
	2		68.3	20.3	6.6	13.2	0.5
	4		67.5	21.1	2.7	17.7	0.7
	6		66.3	22.3	5.2	16.7	0.4
A. aerogenes	0	88.6	62.6	26.0	9.6	16.3	0.1
	2		75.5	13.1	0.2	11.8	1.1
	4		75.5	13.1	0.9	11.9	0.3
	6		71.7	16.9	1.3	14.8	0.8
Intermediate	0	88.6	62.6	26.0	9.6	16.3	0.1
	2		71.5	17.1	1.7	15.2	0.2
	4		68.9	19.7	3.2	16.3	0.2
	6		68.7	19.9	2.3	17.5	0.1

and six days of incubation. The results are presented in table 2. The data for protein nitrogen represent the difference between total and non-protein nitrogen. "Polypeptide" nitrogen was calculated in the manner employed by Kendall and Bly (1922),

mentioned previously. Connor, Peterson and Riker (1937) consider "difference" nitrogen a more accurate term for this fraction, since, in addition to peptide nitrogen, it also includes non-amino nitrogen of amino acids and other undetermined nitrogen. It is apparent that with *E. coli* the greatest change in nitrogen distribution occurred in this fraction, thus confirming the report of Kendall and Bly. The increase of amino nitrogen in *E. coli* cultures between the second and fourth days appeared to be characteristic, as it was found in several experiments. It may be taken to indicate hydrolysis of polypeptides to simpler peptides or to amino acids.

A. aerogenes caused the greatest change in soluble or non-protein nitrogen, reducing its concentration in the medium nearly 50 per cent in two days. This organism likewise caused the greatest decrease in amino nitrogen. Almost all the difference nitrogen was removed from solution in the first two days of growth.

The intermediate culture likewise utilized almost all the difference nitrogen within two days, but caused little change in amino nitrogen.

In every case, the data indicate a considerable synthesis of bacterial protein during the first two days of growth. Thereafter the protein nitrogen decreased while difference nitrogen, and in some cases amino nitrogen, increased. It is therefore evident that cellular autolysis took place. It is interesting to note that in no case were large amounts of ammonia liberated.

UTILIZATION OF AMMONIA IN PRESENCE OF PEPTONE

Experiments were performed in which the culture medium contained both ammonium chloride and peptone as sources of nitrogen, in an attempt to determine which form of nitrogen is more readily utilized by coliform bacteria. The results of analyses are shown in table 3. In general, the changes in nitrogen distribution are similar to those shown in the previous table, when peptone alone served as nitrogen source. As before, the greatest changes occurred in the difference nitrogen fraction. In no case was ammonia utilized to any appreciable extent. In fact, there

was an increase of ammonia nitrogen in every culture at some time during the period of incubation.

It must be remembered that the ammonia content of the medium at any moment may represent merely the balance between the processes of production and utilization. That is, an organism may liberate ammonia by deamination of amino acids and immediately utilize it as source of nitrogen. Accumulation

TABLE 3

Utilization of peptone and ammonia nitrogen by coliform bacteria

Medium: peptone (0.5 per cent), NH_4Cl (0.075 per cent), glucose (0.5 per cent), K_2HPO_4 (0.5 per cent); pH 7. Nitrogen in milligrams per 100 ml.

CULTURE	AGE	TOTAL N	PROTEIN N	NON-PROTEIN N	DIFFERENCE N	AMINO N	AMMONIA N
	days						
E. coli	0	102.6	60.3	42.3	8.0	14.6	19.7
	2		65.1	37.5	3.9	13.2	20.4
	4		63.7	38.9	0.2	18.6	20.1
	6		62.6	40.0	4.8	14.7	20.5
A. aerogenes	0	102.6	60.3	42.3	8.0	14.6	19.7
	2		68.4	34.2	2.5	11.3	20.4
	4		71.4	31.2		11.1	
	6		72.5	30.1	2.5	9.5	18.1
Intermediate	0	102.6	60.3	42.3	8.0	14.6	19.7
	2		66.3	36.3	2.3	15.9	18.1
	4		64.6	38.0	3.8	14.6	19.6
	6		64.0	38.6	7.8	10.7	20.1

of ammonia in the medium should occur only in the absence of a suitable supply of available energy.

It is possible that amino acids or simple peptides may be synthesized directly into bacterial protoplasm without intermediate deamination. Wilson, Peterson and Fred (1930) showed that there was strong possibility of synthesis of protoplasm from amino acids by an anaerobe, *Clostridium acetobutylicum*. The fact that in the present experiments the ammonia content of the medium did not decrease indicates that this may also be

true for some of the coliform bacteria. If the organisms were deaminating amino acids and then using the resulting ammonia to build up protoplasm, it is reasonable to assume that they might also utilize some of the ammonia which was added as ammonium chloride, and which could be secured without the expenditure of energy necessary to split it off from amino acids. There should consequently result a net decrease in ammonia nitrogen. It may be objected that some more "active" form of ammonia, such as might result in the process of deamination, is required for bacterial synthesis. This, however, is contradicted by the demonstrated ability of coliform bacteria to utilize nitrogen from ammonium salts. Therefore, the data strongly indicate that amino acids or simple peptides may be assimilated directly by these bacteria.

Since it was evident that the coliform cultures studied made greatest use of organic nitrogen even in the presence of added ammonia, and since the greatest change in nitrogen distribution occurred in the difference or "polypeptide" fraction, an experiment was performed to give further evidence on this point by determining soluble peptide nitrogen directly. Alpha-amino nitrogen was determined as usual on tungstic acid filtrates of the cultures, both before and after acid hydrolysis. In general, the nitrogen distributions corresponded with those already reported. Relatively great changes were noted in the peptide fraction, thus confirming the trend of the calculated difference nitrogen data.

UTILIZATION OF GLUCOSE AND NON-PROTEIN NITROGEN

The relationship between growth, utilization of glucose and decrease in tungstic acid-soluble or non-protein nitrogen is shown by the following experiment. A medium containing peptone (0.5 per cent), glucose (0.4 per cent), K_2HPO_4 (0.1 per cent), at pH 7, was employed. This was dispensed in bottles containing 30 ml. of 1 per cent agar containing 5 per cent of $CaCO_3$ to neutralize acids produced during the fermentation. Plate counts, glucose and tungstic-acid-soluble nitrogen determinations were

made immediately after inoculation, and again after incubation at 37°C. for twelve and twenty-four hours. The results are shown in table 4.

The data show that, with respect to the first twelve hours of growth, the *cells* of *A. aerogenes* were much more active than those of *E. coli* in the utilization of glucose and non-protein nitrogen (the decrease in the latter per milliliter of culture was not as great, however, with *Aerobacter* as with *Escherichia*). The ratio of glucose to non-protein nitrogen shows that the *Aerobacter* strain utilized more glucose per milligram of nitrogen than did the *Escherichia*. This may indicate that the former organism required more carbon either for cellular materials, or as source of energy for metabolic processes, or both. The results of the twenty-four hour analyses show that upon exhaustion of the carbohydrate, the ratio of glucose to non-protein nitrogen fell, as would be expected.

TABLE 4

Utilization of glucose and non-protein nitrogen by coliform bacteria

Initial analysis: glucose = 3.63 mgm. per ml.; non-protein (tungstic acid soluble) nitrogen = 0.289 mgm. per ml.

AGE	E. COLI		A. AEROGENES		INTERMEDIATE
	12 hours	24 hours	12 hours	24 hours	12 hours
Increase in bacterial count per ml. ($\times 10^8$)	755	855	486	796	168
Glucose utilized (mgm. per ml.)	2.76	3.49	3.27	3.40	1.57
Glucose utilized per cell (mgm. $\times 10^{-9}$)	3.66	4.08	6.73	4.27	9.35
Non-protein N utilized (mgm. per ml.)	0.057	0.081	0.050	0.091	0.032
Non-protein N utilized per cell (mgm. $\times 10^{-11}$)	7.55	9.48	10.29	11.42	19.05
Ratio: $\frac{\text{Glucose}}{\text{Non-protein N}}$	48.4	43.1	65.5	37.4	48.1

The decrease was much greater in the case of *A. aerogenes* then in that of *E. coli*, and roughly paralleled the increase in bacterial count. This indicates attack upon the nitrogenous

constituent of the culture medium as source of food and energy, particularly in the *Aerobacter* culture. Analysis of cultures of the intermediate strain after twelve hours of growth showed greater utilization *per cell* of glucose and non-protein nitrogen than was obtained with either of the other two strains, but the glucose: non-protein nitrogen ratio was practically identical with that of *E. coli*.

DISCUSSION

It has been found that strains of *Escherichia*, *Aerobacter*, and an *Escherichia-Aerobacter* intermediate possessed the ability to utilize ammonia nitrogen in the absence of an organic source, but that when peptone nitrogen also was present there was no apparent utilization of ammonia nitrogen. In the peptone medium there was evidence of attack on the soluble or non-protein nitrogen, particularly the difference or "polypeptide" fraction. The strain of *Aerobacter aerogenes* showed greater activity than did the other two coliform strains in its action on soluble and amino nitrogen. This may be correlated in part with the rapid utilization of glucose by this organism and consequent necessity for obtaining carbon and energy from some other source, e.g., peptone.

The so-called sparing action of carbohydrates on protein utilization has been emphasized by Berman and Rettger (1918b), Kendall *et al.*, (1922), and by others. In particular, it has been noted that, in the absence of carbohydrates, there is a tendency toward increased ammonia production, attributed to deamination of amino acids. However, tables 2 and 3 indicate that certain bacteria may assimilate amino and simple peptide nitrogen directly and build it into protoplasm. This is noted particularly in the case of the *Aerobacter aerogenes* cultures, in which glucose utilization was practically complete after the first day (*cf.* table 4). These showed a decrease of amino nitrogen far in excess of the change in ammonia nitrogen. That some constituent of the soluble or non-protein fraction of nitrogen was increasingly attacked after exhaustion of the carbohydrate was shown in table 4, where it was noted that *Aerobacter aerogenes*, in particular, evidently made use of soluble nitrogenous material as source of carbon and energy.

SUMMARY

Strains of *Escherichia*, *Aerobacter*, and an *Escherichia-Aerobacter* intermediate utilized ammonia when this was supplied as a sole source of nitrogen. However, when peptone nitrogen also was present, there was no apparent utilization of ammonia.

Periodic analyses indicated that amino and simple peptide nitrogen might be assimilated directly and built into protoplasm without intermediate deamination.

The author is greatly indebted to Dr. W. H. Peterson for suggestions and helpful criticism during the course of the work.

REFERENCES

BERMAN, N., AND RETTGER, L. F. 1918a Bacterial nutrition: Further studies on the utilization of protein and non-protein nitrogen. Jour. Bact., **3**, 367–388.

BERMAN, N., AND RETTGER, L. F. 1918b The influence of carbohydrate on the nitrogen metabolism of bacteria. Jour. Bact., **3**, 389–402.

BRAUN, H., AND CAHN-BRONNER, C. E. 1921 Die synthetischen Fähigkeiten verschiedener Bakterienarten. Zent. f. Bakt. I Orig., **86**, 196–211.

CONNOR, H. A., PETERSON, W. H., AND RIKER, A. J. 1937 The nitrogen metabolism of the crown gall and hairy root bacteria. Jour. Agric. Res., **54**, 621–628.

KENDALL, A. I., AND BLY, R. S. 1922 The nitrogenous metabolism of *Bacillus coli*. Studies in bacterial metabolism, LXII. Jour. Inf. Dis., **30**, 239–244.

KENDALL, A. I., AND HANER, R. C. 1922 Studies in bacterial metabolism, LVIII–LXI. *B. dysenteriae* (Shiga), *B. typhosus*, *B. paratyphosus alpha*, *B. paratyphosus beta*. Jour. Inf. Dis., **30**, 225–238.

KNIGHT, B. C. J. G. 1936 Bacterial nutrition. Med. Res. Council, London, p. 52.

KOSER, S. A. 1918 The employment of uric acid synthetic medium for the differentiation of *B. coli* and *B. aerogenes*. Jour. Inf. Dis., **23**, 377–379.

KOSER, S. A., AND RETTGER, L. F. 1919 Studies on bacterial nutrition. The utilization of nitrogenous compounds of definite chemical composition. Jour. Inf. Dis., **24**, 301–321.

MITCHELL, N. B. 1937 Characteristics and differentiation of the "intermediates" of the colon-aerogenes group of bacteria. Thesis, Iowa State College.

WILSON, P. W., PETERSON, W. H., AND FRED, E. B. 1930 The relationship between the nitrogen and carbon metabolism of *Clostridium acetobutylicum*. Jour. Bact., **19**, 231–260.

CERTAIN ELECTROPHORETIC RELATIONSHIPS OF COLON BACILLI

GEORGE H. CHAPMAN AND CLARENCE W. LIEB

Clinical Research Laboratory, New York, New York

Received for publication June 15, 1938

Many investigators have shown that the electrophoretic migration velocities of different bacteria are parallel with certain other properties of the cultures. The relationships to be described were noted during the course of an investigation of the electrophoretic migration velocities of colon bacilli.

The technic of Chapman (1929) was employed, the capillary cell which was described by Falk, Jensen and Mills (1928) being used because it is convenient for studying a large number of cultures. Electrophoretic determinations were made almost daily over a period of about 5 years, during which time the average velocities of three control strains of *Escherichia coli* remained constant. Tittsler and Berry (1938) found that the velocity of colon bacilli remained constant during serial cultivation on a wide variety of culture media.

In our earlier series, however, the individual measurements of the control strains were not constant. The variations were traced to changes in the distance between the electrodes which, being made of thin platinum wire, sometimes became bent while the capillary tubes were being placed in position or when the cell was being cleaned. Because of the difficulty of adjusting the distance precisely by means of calipers, and of assuring that the electrodes were parallel below the water line, the speed of the strains was controlled by a potentiometer. Before the tests were made each day, the speed of one of the standard strains was adjusted by the potentiometer until it coincided with its average velocity. The dial setting was kept at this point throughout the tests. The other two control strains were tested at the end of the

day's series. If they showed deviations from their average velocities, it indicated that the electrodes had become bent during the tests and the calculations were, therefore, erroneous. This occurred only once, and the results were discarded.

When suitably controlled, the results by this method should be comparable with those obtained by instruments which permit calculation of the absolute zeta potential. The results obtained

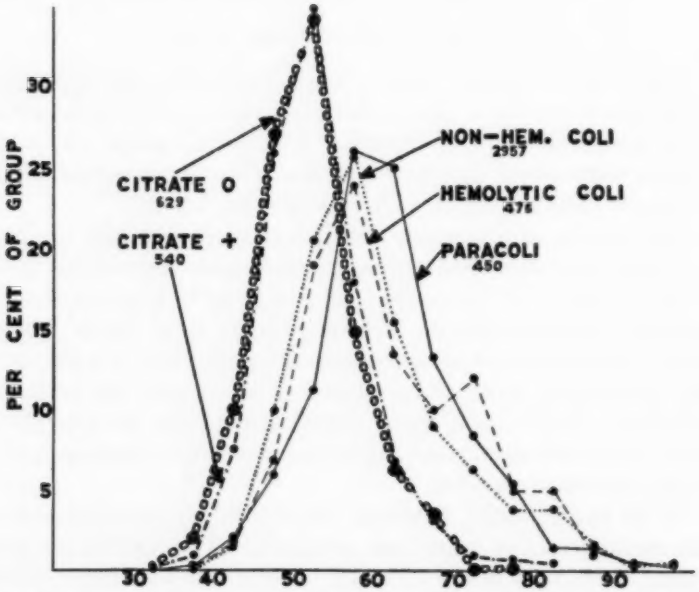


FIG. 1. FREQUENCY DISTRIBUTION OF ELECTROPHORETIC MOBILITIES OF 5052 COLI-AEROGENES CULTURES

with the second control strain did not vary more than 2 per cent from the calculated average. The observed velocities of the third strain, which was more difficult to calculate because of its excessive speed, did not vary more than 4 per cent from the calculated average. Dozois, *et al.* (1932), Rosenow (1934) and Chapman (1929) agree that such readings in general do not vary more than 4 per cent.

Every effort was made to prevent contamination of the apparatus by grease. The capillary tubes were boiled several hours in bichromate-sulfuric acid cleaning fluid.

The velocities of 5052 cultures of colon bacilli were determined as just described and the results are classified in figure 1. The frequencies of citrate-utilizing and non-utilizing strains which produced an *Aerogenes* type of colony on Levine's eosine methylene-blue medium were identical, the modes being 53μ/100

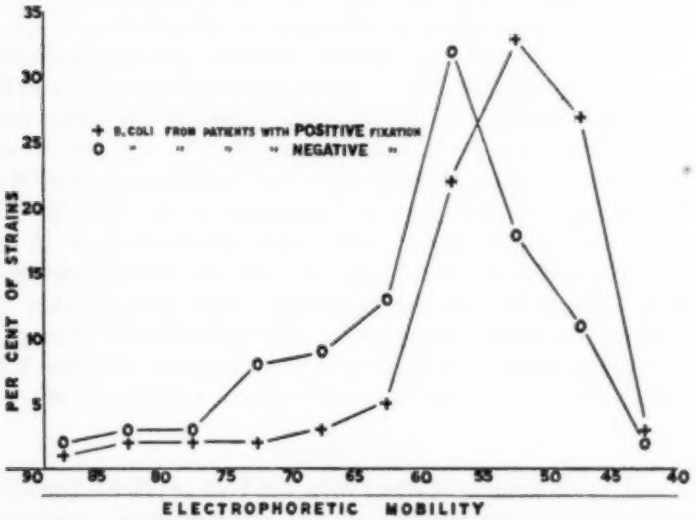


FIG. 2. RELATIONSHIP BETWEEN THE ELECTROPHORETIC MIGRATION VELOCITIES OF *E. coli* STRAINS AND THE COMPLEMENT-FIXING POWER WITH *E. coli* ANTIGEN OF THE SERUM FROM THE PERSONS FROM WHOM THE STRAINS WERE ISOLATED

applied volts/sec./3.45 cm. The frequencies of hemolytic and non-hemolytic strains of *E. coli* were similar, the modes being about 58μ. Paracoli (non-lactose fermenters, or degraded colon bacilli) had a mode of about 58 to 63μ. Dozois (1936) found the average velocity of *Escherichia* to be different from the average of *Aerobacter*.

Of 1237 strains which were considered as having produced an *Aerogenes* type of colony on Levine's eosine methylene-blue

medium, 55.5 per cent did not utilize citrate on Simmons' medium. The taxonomic position of these strains has not been determined. Their failure to utilize citrate, and their reactions to indol, methyl-red and Vosges-Proskauer tests suggests that they belong to the genus *Escherichia*. Mucoid forms of *E. coli* were described by Parr (1933). However, the electrophoretic frequency distribution curves and the appearance of the colonies on Levine's medium indicate that they may be more closely related to *Aerobacter* than to *E. coli*. Complement-fixation reactions support this view, as will be shown elsewhere.

There was a general relationship between the electrophoretic migration velocities of *E. coli* strains isolated from feces and the complement fixing power of the active serum of the person from whom the cultures had been isolated (fig. 2). Of the *E. coli* strains isolated from persons whose sera fixed complement with *E. coli* antigen, 63 per cent had velocities of less than 52μ/100 applied volts/sec./3.45 cm., while only 31 per cent of *E. coli* strains from persons whose sera did not fix complement with *E. coli* antigen had similar velocities. This suggests that the power to stimulate complement-fixing antibodies is related to the electrophorectic mobilities of the cultures; possibly both characteristics are in part dependent upon a common factor.

CONCLUSIONS

Based upon results obtained with the Falk capillary type cell, the distribution frequencies of the electrophoretic migration velocities of cultures producing an *Aerogenes* type of colony on Levine's eosine methylene-blue medium were similar, regardless of their reactions to indol, methyl-red and Vosges-Proskauer tests, but were different from those of *Escherichia coli* and non-lactose fermenters.

Strains of *E. coli* isolated from persons whose sera fixed complement with *E. coli* antigen had a different frequency distribution of electrophoretic migration velocities than had strains isolated from persons whose sera did not fix complement with *E. coli* antigen. This suggested that differences in electrophoretic migration velocities of *E. coli* cultures are associated with differ-

ences in the power of the cultures to stimulate the production of complement fixing antibodies to *E. coli*.

REFERENCES

CHAPMAN, G. H. 1929 Electrophoretic potential as an aid in identifying strains of the *B. coli* group. Jour. Bact., **18**, 339–342.

DOZOIS, K. P., TITTSLER, R. P., LISSE, M. W., AND DAVEY, W. P. 1932 Effect of "low voltage" x-rays on electrophoretic migration velocity, viability and pH of *Escherichia coli* suspensions. Jour. Bact., **24**, 123–132.

DOZOIS, K. P. 1936 Variations in the electrophoretic mobilities of Escherichia, Aerobacter and "intermediate" strains. Jour. Bact., **31**, 211–215.

FALK, I. S., JENSEN, L. B., AND MILLS, J. H. 1928 Electrophoresis of diphtheria bacilli. III. The development of a simplified technique for electrophoretic measurement of the virulence of diphtheria bacilli. Jour. Bact., **15**, 421–450.

PARR, L. W. 1933 Mucoid *Bacterium coli* in feces of normal subject. Proc. Soc. Exper. Biol. & Med., **31**, 226–227.

ROSENOW, E. C. 1934 Cataphoretic velocity and virulence of streptococci isolated from throats of human beings, from raw milk, flies, water, sewage and air during epidemics of common autumnal cold. Amer. Jour. Hygiene, **19**, 1–21.

TITTSLER, R. P., AND BERRY, G. P. 1938 The electrophoretic migration velocity of *Escherichia coli* after cultivation on media of varying composition. Jour. Bact., **35**, 213–222.

THE SPECIFICITY OF THE AGGLUTININ REACTION FOR SHIGELLA DYSENTERIAE

II. Agglutinin Absorption Relationships Between Shigella Dysenteriae and Escherichia Coli

THOMAS T. MACKIE[1]

With the Assistance of Mildred Schweiger

Department of Public Health and Preventive Medicine, Cornell University Medical College; and The Gray Laboratory, The Roosevelt Hospital, New York City

Received for publication June 30, 1938

In a previous communication (Mackie, 1938) we have reported observations upon the *Shigella dysenteriae* agglutinin curves in a group of patients presenting the intestinal pathology of chronic bacillary dysentery and bacteriologic or serologic evdence of infection by these organisms. Fluctuation of the titre curves in individual patients, the common finding of higher titres in the culture-negative cases than in the culture-positive cases, and the infrequency and low titre of normal agglutinins in the control groups, suggest that agglutinins for *Shigella dysenteriae* may develop in response to non-specific heterologous infection. The studies here reported were undertaken to investigate this possibility.

It is known that immunization of an animal against *Eberthella typhosa* often leads to the production of major agglutinins for the homologous organism, and of minor agglutinins for certain strains of *Escherichia coli*. Absorption of such a serum by its homologous antigen removes the minor as well as the major agglutinins (Bulloch, 1929). Agglutinins for *S. dysenteriae*, previously absent, have been observed to appear in the serum of certain patients suffering from chronic ulcerative colitis in the

[1] Associate, Department of Public Health and Preventive Medicine, Cornell University Medical College; Attending Physician, The Roosevelt Hospital.

course of immunization with a mixture of *E. coli* toxin and vaccine (Winkelstein).

According to Gay (1935) it is not unusual, in bacillary dysentery, to recover *E. coli* which agglutinate in Flexner anti-serum. This phenomenon of para-agglutination was first reported by Duval and Shorer (1904). It was confirmed subsequently by others (Park and Williams, 1910; Kuhn, Gildemeister and Warthe, 1911; Kligler, 1918).

Hegedus (1933) has reported the isolation of strains of *E. coli* from dysentery patients which were agglutinated by Flexner antiserum. An anti-coli serum prepared with one of these strains agglutinated Flexner bacilli to fifty per cent titre. Reciprocal absorption of this serum by the homologous and heterologous bacilli completely removed the agglutinins for both organisms. It is not entirely clear, however, that he was using uniformly smooth cultures. Rough variants of several Flexner strains and Sonne bacilli have been shown to be related to a group of non-hemolytic, non-sucrose-fermenting *E. coli* (White, 1929).

A wide immunological relationship exists between the "R" variants of the intestinal bacilli (Schütze, 1921; White, 1926; Arkwright, 1931). Moreover, the "O" (somatic) antigen in any group is shared by many organisms in that group, and occasionally by unrelated organisms (Zinsser and Bayne-Jones, 1935). The agglutination relationships between rough variants of the intestinal bacilli are determined by the "O" antigens and agglutinins of roughness. Partial "R" dissociation, therefore, may lead to non-specific agglutination. Thibault and Braunberger (1935), working with the Shiga bacillus, have found that neither colony appearance nor physical tests could be depended upon to insure pure "S" strains.

MATERIAL

We have isolated thirteen strains of *Escherichia coli* which exhibit close antigenic relationship to *Shigella dysenteriae* Sonne and Flexner. These were obtained from twelve patients whose sera agglutinated Flexner or Sonne strains but were repeatedly negative on culture at proctoscopy for *S. dysenteriae*. The ag-

glutinability of these strains of *E. coli*, and their ability to absorb the dysentery agglutinins from the patients' sera have been investigated. In like fashion their behavior in monovalent anti-dysentery rabbit serum has been studied. Each strain of *E. coli* has been used to produce a monovalent anti-coli rabbit serum, and the agglutinability and agglutinin absorption capacity of the homologous *E. coli* and heterologous *S. dysenteriae* have been studied.

METHODS

Care was exercised to insure the use of smooth strains of *E. coli* and of *S. dysenteriae* for the preparation of anti-sera, for the absorption antigens, and for the preparation of formolized antigens for agglutination tests. Repeated plate subcultures were used. Discrete colonies were examined under high magnification with the dissecting microscope. Only smooth glassy colonies were replated. These replatings were continued until subcultures were uniformly smooth. One smooth colony was then fished to slants for preparation of the antigen, and plate cultures were made from the slant growth and re-examined for smoothness. All were tested for spontaneous flocculation in 0.85-per-cent sodium chloride solution.

Agglutination reactions have been performed with formolized antigens. Standard agglutination tubes have been used, and in each instance the end point of the reaction has been considered to be that dilution in which definite although incomplete clumping is visible to the unaided eye. The detailed technique was identical with that used in the previous study (Mackie, 1938).

Living antigens were used throughout for absorbing the sera. In each instance the serum was diluted 1/5 with 0.85 per cent saline solution. To this was added an equal volume of cell suspension. For absorbing the human sera, a 1/20 cell dilution was used. Lack of sufficient serum made titration impracticable. For the rabbit sera, however, the absorbing dose was titrated in each instance and that dose chosen which reduced the homologous agglutinin titre to 1/20. These cell dilutions varied from 1/2 to 1/25. The suspension of living cells and diluted serum was

held in the water bath at 45°C. for two hours, and then placed in the icebox over night. The serum and bacterial bodies were separated by centrifuge and the supernatant fluid tested in serial dilution with formolized antigen.

The *Escherichia coli* strains were recovered by direct inoculation at proctoscopy upon plates of MacConkey's medium, eosin methylene-blue and desoxycholate agar. After twenty-four hours incubation, one hundred random colonies were fished to rabbit blood agar plates. These were kept at room temperature over night. Five nonhemolytic, and, if present, five hemolytic colonies, were inoculated by stab into tubes of 0.5 per cent hormone agar and thence to agar slants. These ten strains were then tested against the monovalent anti-dysentery rabbit sera. All those that agglutinated were retained and one non-agglutinating strain was likewise retained for purposes of control.

The cultural characteristics of the *Escherichia coli* are shown in table 1. Five were motile. Eight were non-motile. Ten of the strains were hemolytic on rabbit-blood agar. All were gas formers. All gave positive methyl-red and negative Voges-Proskauer reactions. Gelatin was not liquefied. Indol was tested for colorimetrically. 5 cc. broth cultures containing 1 per cent peptone and 0.5 per cent NaCl were incubated at 37.5°C. for two days. To these were then added 1 cc. dimethylamido benzaldehyde (2 grams in 100 cc. ethyl alcohol) and 6–7 drops of concentrated HCl. The mixture was then shaken with 2 cc. of chloroform and the color of the latter checked against LaMotte pH indicators. The presence of indol is demonstrated by a pH reading ranging from 7.2 to 8.2

The *Shigella dysenteriae* strains are subcultures of known types (Murray, 1918) kindly furnished to us by Professor E. G. D. Murray of McGill University, as follows:

PB24, Karim Kahn: Y Ledingham, Mannitol fermenting; Flexner types; E. Ledingham: Slow lactose-fermenting; Sonne.

These have been compared with strains of *S. dysenteriae* obtained from the Hygienic Laboratory of the United States Public Health Service. The cultural reactions are comparable (table 2). Agglutination and agglutinin absorption tests confirm the classifi-

TABLE 1

Cultural characteristics 13 strains Escherichia coli

NAME	MOTILITY	HEMOLYSIS	GLUCOSE	LACTOSE	MANNITOL	MALTOSE	SUCROSE	XYLOSE	DULCITOL	MILK	INDOL pH*	METHYL RED	VOGES PROSKAUER	GELATIN LIQUEFACTION
MI-H6	−	+	A+G	A+G	A+G	A+G	A+G4	A+G	A+G	A+C	7.9	+ weak	−	−
VC-H3	−	+	A+G	A+G	A+G	A+G	A+G	A+G	A+G	A+C	7.9	+ weak	−	−
S-H6	+	+	A+G	A+G	A+G	A+G	A+G5	A+G	A+G	A+C	7.6	+ weak	−	−
G-NH$_3$	−	−	A+G	A+G	A+G	A+G	ALK	A+G	A+G	A+C	7.8	+ weak	−	−
S-NH	−	−	A+G	A+G	A+G	A+G	ALK	A+G	ALK	A+C	7.8	+ weak	−	−
W-NH$_7$	+	−	A+G	A+G	A+G	A+G	A+G3	A+G	A+G	A+C3	8.1	+ weak	−	−
W-H$_7$	+	+	A+G	A+G	A+G	A+G	A+G7	A+G	A+G	A+C3	7.9	+ weak	−	−
T-H$_1$	−	+	A+G	A+G	A+G	A+G	ALK	A+G	A+G5	A+C	7.8	+ strong	−	−
A-H6	+	+	A+G	A+G	A+G	A+G	ALK	ALK	A+G	A+C	7.8	+ moderate	−	−
W-H12	+	+	A+G	A+G	A+G	A+G	ALK	A+G	A+G	A+C	8.2	+ weak	−	−
L-H2	−	+	A+G	A+G	A+G	A+G	ALK	A+G	A+G	A+C	7.9	+ moderate	−	−
L-H1	−	+	A+G	A+G	A+G	A+G	A+G5	A+G	A+G	A+C	7.2	+ weak	−	−
G-H2	−	+	A+G	A+G	A+G	A+G	A+G5	A+G	A+G	A+C	7.9	+ weak	−	−

* Positive indol reaction—pH 7.2 to 8.2.

Symbols: A+G = Acid and gas, 24 hours incubation.
A+G4 = Acid and gas, 4 days incubation.
ALK = Alkaline.
A+C = Acid and clot, 24 hours incubation.
A+C3 = Acid and clot, 3 days incubation.

TABLE 2

NAME	MOTILITY	GLUCOSE	LACTOSE	MANNITOL	MALTOSE	SUCROSE	XYLOSE	DULCITOL	MILK	INDOL pH†	METHYL RED	VOGES PROSKAUER	GELATIN LIQUEFACTION
Cultural characteristics—S. dysenteriae antigens													
E. Ledingham	–	A	A-6*	A	A	A-4	A	ALK	AC-14	7.6	–	–	–
PB 24	–	A	ALK	A	A	ALK	ALK	ALK	A-ALK	7.4	+	–	–
Karim Kahn	–	A	ALK	A	A	ALK	ALK	ALK	A2-ALK	7.5	+	–	–
Y Ledingham	–	A	ALK	A	ALK	ALK	ALK	ALK	A-ALK	7.6	+	–	–
Comparable strains of S. dysenteriae													
Dispar #29	–	A	A-16	A	A	A-4	A	ALK	AC-16	7.7	+	–	–
Flexner #57	–	A	ALK	A	A	ALK	ALK	ALK	ALK	–	+	–	–
VX #37	–	A-3	ALK	A-5	ALK	ALK	ALK	ALK	ALK	–	–	–	–
Flexner Lister V	–	A	ALK	A	A	ALK	ALK	ALK	ALK	7.9	+	–	–
Y Ledingham #44	–	A	ALK	A	ALK	ALK	ALK	ALK	ALK	8.0	–	–	–

* Numeral indicates days of incubation at 37.5°C.

† Positive indol reaction—pH 7.2 to 8.2.

cation of the antigens used for this and the preceding study. Dispar #29 agglutinates to full titre of the E. Ledingham serum and completely absorbs the homologous agglutinins. Flexner #57 and VX #37 agglutinate to full titre in PB24 serum and completely remove the homologous agglutinins. Flexner Lister V agglutinates to the full titre of Karim Kahn antiserum and completely absorbs the homologous agglutinins. Y Ledingham #44 agglutinates to full titre of our Y Ledingham serum and completely removes the homologous agglutinins.

EXPERIMENTAL DATA

Findings with patients' sera

Sera from the twelve patients from whom the *Escherichia coli* were recovered were tested for agglutinins against the homologous *E. coli* and the four *S. dysenteriae* antigens (table 3). The titres for the latter ranged from 1/80 to 1/640. Five of the coli strains failed to agglutinate; eight showed agglutination at dilutions of 1/40 to 1/640. In four instances the coli and the dysentery agglutinin titres were identical. One strain of *E. coli* agglutinated in higher dilution than the *S. dysenteriae* antigens.

Nine of the sera, after absorption with the corresponding strains of *E. coli*, showed complete disappearance of agglutinins for one or more of the strains of *S. dysenteriae*.

Five of the sera, after absorption with one or more of the *S. dysenteriae* antigens, showed complete loss of agglutinins for the corresponding strain of *E. coli*. In one serum the titre was reduced and in one it was unchanged. Lack of serum prevented studies of the remaining sera.

Findings with monovalent anti-dysentery rabbit sera

Four monovalent rabbit anti-sera were used for this study. PB24, Karim Kahn, and E. Ledingham reached a titre of 40,960. the Y Ledingham serum titre was 20,480.

The agglutinability of the thirteen strains of *E. coli* was studied in these sera unabsorbed, after absorption by the homologous *S. dysenteriae*, and after absorption by the heterologous *E. coli*. In each instance absorption by the homologous antigen removed

TABLE 3

Agglutination titres S. dysenteriae and homologous E. coli in patients' serum

PATIENT	STRAIN E. COLI	TITRE FOR S. DYSENTERIAE	TITRE FOR HOMOLOGOUS E. COLI	SERUM ABSORBED WITH HOMOLOGOUS E. COLI		SERUM ABSORBED WITH S. DYSENTERIAE		
				Final titre E. coli	Final titre S. dysenteriae	Absorbing organism	Final titre for corresponding S. dysenteriae	Final titre for E. coli
MI	H6	E. Led 1 + 1/640 Y. Led 1 + 1/640 KK 4 + 1/320	1 + 1/640	0	E. Led 0 Y. Led 1 + 1/320 KK 1 + 1/320	E. Led Y. Led KK	0 0 0	0 4 + 1/640 4 + 1/640
VC	H3	E. Led 1 + 1/640 PB24 1 + 1/160	1 + 1/320	0	E. Led 0 PB24 0			
S	H6	E. Led 1 + 1/640 PB24 4 + 1/160 KK 1 + 1/160 Y. Led 1 + 1/640	4 + 1/640	0	E. Led 4 + 1/40 PB24 4 + 1/160 KK 3 + 1/160 Y. Led 1 + 1/640	E. Led PB24 KK Y. Led	0 1 + 1/80 1 + 1/160 1 + 1/320	0 4 + 1/320 4 + 1/640 4 + 1/640
G	NH3	E. Led 1 + 1/160 PB24 1 + 1/160 KK 1 + 1/160 Y. Led 1 + 1/160	1 + 1/320	0	E. Led 1 + 1/80 PB24 1 + 1/160 KK 1 + 1/160 Y. Led 1 + 1/160	E. Led PB24 KK Y. Led	0 0 0 0	1 + 1/320 1 + 1/320 1 + 1/320 1 + 1/320
S	NH	E. Led 1 + 1/640 KK 4 + 1/640 Y. Led 4 + 1/160	1 + 1/320	0	E. Led 1 + 1/320 KK 0 Y. Led 0	E. Led KK Y. Led	0 0 0	1 + 1/320 0 1 + 1/160
W	NH7	E. Led 1 + 1/320 PB24 1 + 1/160 KK 4 + 1/80 Y. Led 4 + 1/160	0	0	E. Led 1 + 1/320 PB24 1 + 1/80 KK 0 Y. Led 1 + 1/160	Y. Led	0	

W	H7	E. Led 1 + 1/320 PB24 1 + 1/160 KK 4 + 1/80 Y. Led 4 + 1/160	0	0	E. Led 1 + 1/320 PB24 1 + 1/80 KK 4 + 1/80 Y. Led 1 + 1/160	KK	0	
T	H1	E. Led 1 + 1/160 KK 4 + 1/160 Y. Led 1 + 1/160	1 + 1/160	0	E. Led 0 KK 1 + 1/160 Y. Led 1 + 1/80	E. Led KK Y. Led	0 0 0	0 1 + 1/320 1 + 1/320
A	H6	E. Led 1 + 1/80 PB24 1 + 1/80 KK 1 + 1/160 Y. Led 1 + 1/160	2 + 1/40	0	E. Led 3 + 1/20 PB24 2 + 1/20 KK 2 + 1/40 Y. Led 1 + 1/80	E. Led PB24 KK Y. Led	0 0 0 0	1 + 1/40 1 + 1/40 1 + 1/40 1 + 1/40
W	H12	E. Led 4 + 1/160 KK 4 + 1/160 Y. Led 2 + 1/160	0	0	E. Led 0 KK 4 + 1/80 Y. Led 4 + 1/160	E. Led	0	
L	H2	E. Led 4 + 1/160 PB24 1 + 1/160 KK 1 + 1/160 Y. Led 1 + 1/320	1 + 1/320	0	E. Led 0 PB24 1 + 1/80 KK 1 + 1/160 Y. Led 1 + 1/160	E. Led PB24 KK Y. Led	0 0 0 0	0 4 + 1/320 1 + 1/320 4 + 1/320
L	H1	E. Led 1 + 1/160	0	0	E. Led 0			
G	H2	E. Led 1 + 1/160	0	0	E. Led 0			

both the homologous dysentery agglutinins and the heterologous coli agglutinins. Absorption of the sera by the individual *E. coli* strains removed the corresponding coli agglutinins.

Unabsorbed PB24 serum did not agglutinate any of the *E. coli* strains to full titre (table 4). One strain, S-NH, reached half titre, and two, W-NH and W-H7, agglutinated at one-eighth titre. All the remaining strains agglutinated in serum dilutions varying from 1/160 to 1/1280.

TABLE 4

Agglutinin titres—E. coli vs. PB24 serum

PB24 SERUM UNABSORBED		PB24 ABSORBED WITH PB24	PB24 SERUM ABSORBED WITH E. COLI VS. E. COLI		PB24 SERUM ABSORBED WITH E. COLI VS. PB24	
			Absorbing organism	Final titre	Absorbing organism	Final titre
PB24	40,960	20				
MI-H6	2,560	20	MI-H6	20	MI-H6	40,960
VC-H3	160	20	VC-H3	20	VC-H3	40,960
S-H6	320	20	S-H6	20	S-H6	40,960
G-NH3	1,280	20	G-NH3	20	G-NH3	10,240
S-NH	20,480	20	S-NH	20	S-NH	5,120
W-NH	5,120	20	W-NH	20	W-NH	40,960
W-H7	5,120	20	W-H7	20	W-H7	10,240
T-H1	1,280	20	T-H1	20	T-H1	20,480
A-H6	1,280	20	A-H6	20	A-H6	10,240
W-H12	160	20	W-H12	20	W-H12	40,960
L-H2	320	20	L-H2	20	L-H2	20,480
L-H1	160	20	L-H1	20	L-H1	40,960
G-H2	320	20	G-H2	20	G-H2	10,240

Absorption with the individual coli strains variously affected the titre for the homologous dysentery antigen. Strain S-NH, which agglutinated to highest titre in the unabsorbed serum, proved to be the most active absorber of the group, reducing the titre for PB24 to 5,120. W-H7, which agglutinated to an eighth titre, reduced the homologous agglutinins to 10,240. The three strains, G-NH3, T-H1, and A-H6, likewise showed active absorption. They reduced the titre for the homologous PB24 antigen to 10,240, 20,480, and 10,240 respectively. L-H2 and G-H2, although agglutinating only to 1/320, reduced the titre

for the homologous dysentery antigen to 20,480 and 10,240 respectively.

The unabsorbed Karim Kahn serum likewise failed to agglutinate any of the *E. coli* strains to full titre (table 5). Only two, W-H7 and L-H1, reached one-quarter titre. The remainder agglutinated in dilutions varying from 1/320 to 1/5120.

Eight of the *E. coli* strains, when used to absorb the Karim Kahn serum, reduced the titre of this serum for its homologous

TABLE 5

Agglutinin titres—E. coli vs. Karim Kahn serum

KK SERUM UNABSORBED		KK SERUM ABSORBED WITH KK	KK SERUM ABSORBED WITH E. COLI VS. E. COLI		KK SERUM ABSORBED WITH E. COLI VS. KK	
			Absorbing organism	Final titre	Absorbing organism	Final titre
KK	40,960	20				
MI-H6	320	20	MI-H6	20	MI-H6	40,960
VC-H3	640	20	VC-H3	20	VC-H3	40,960
S-H6	640	20	S-H6	20	S-H6	20,480
G-NH3	640	20	G-NH3	20	G-NH3	40,960
S-NH	5,120	20	S-NH	20	S-NH	20
W-NH	2,560	20	W-NH	20	W-NH	5,120
W-H7	10,240	20	W-H7	20	W-H7	5,120
T-H1	1,280	20	T-H1	20	T-H1	20,480
A-H6	2,560	20	A-H6	20	A-H6	10,240
W-H12	640	20	W-H12	20	W-H12	40,960
L-H2	640	20	L-H2	20	L-H2	40,960
L-H1	10,240	20	L-H1	20	L-H1	10,240
G-H2	320	20	G-H2	20	G-H2	10,240

antigen to fifty per cent or less. S-NH, although agglutinating only to 5120, completely absorbed the Karim Kahn agglunitins. G-H2, which agglutinated only to 1/320 in the unabsorbed serum, reduced the titre for the homologous antigen to twenty-five per cent.

The unabsorbed Y Ledingham serum agglutinated one of the *E. coli* strains, W-H7, to full titre; a second, S-NH, to half titre; and a third, W-NH, to one-quarter titre. The remaining ten strains agglutinated at dilutions ranging from 1/160 to 1/2560 (table 6).

Nine of the *E. coli* strains, when used to absorb the Y Ledingham serum, reduced the titre for the homologous dysentery antigen to fifty per cent or less. The completeness of absorption, however, did not parallel exactly the agglutinability of the coli strains in the unabsorbed serum. W-H7, which agglutinated to full titre, reduced the homologous dysentery agglutinins to fifty per cent. S-NH agglutinated at half titre and reduced the homologous agglutinins to 1/1280. W-NH agglutinated at

TABLE 6

Agglutination titres—E. coli vs. Y Ledingham serum

Y LED SERUM UNABSORBED		Y LED SERUM ABSORBED WITH Y LED	Y LED SERUM ABSORBED WITH E. COLI VS. E. COLI		Y LED SERUM ABSORBED WITH E. COLI VS. Y LED	
			Absorbing organism	Final titre	Absorbing organism	Final titer
Y Led	20,480	20				
MI-H6	1,280	20	MI-H6	20	MI-H6	10,240
VC-H3	160	20	VC-H3	20	VC-H3	20,480
S-H6	640	20	S-H6	20	S-H6	10,240
G-NH3	640	20	G-NH3	20	G-NH3	10,240
S-NH	10,240	20	S-NH	20	S-NH	1,280
W-NH	5,120	20	W-NH	20	W-NH	10,240
W-H7	20,480	20	W-H7	20	W-H7	10,240
T-H1	1,280	20	T-H1	20	T-H1	20,480
A-H6	2,560	20	A-H6	20	A-H6	10,240
W-H12	640	20	W-H12	20	W-H12	20,480
L-H2	640	20	L-H2	20	L-H2	5,120
L-H1	160	20	L-H1	20	L-H1	20,480
G-H2	320	20	G-H2	20	G-H2	10,240

twenty-five per cent titre and reduced the dysentery agglutinins to fifty per cent.

Four strains, which agglutinated in relatively low dilutions, 1/320 to 1/640, proved to be active absorbers of Y Ledingham agglutinins, reducing the titre of the serum in these instances to fifty per cent and in one to twenty-five per cent.

The results obtained with E. Ledingham (Sonne) serum were striking (table 7). Six of the coli strains agglutinated at full titre, one at half titre, and two at one-quarter titre. One reached one-eighth titre. The remaining three failed to agglutinate in dilutions above 1/320.

The six strains of *E. coli*, which agglutinated at full titre in the E. Ledingham serum, completely absorbed the dysentery agglutinins. The two strains A-H6 and W-H12, which agglutinated at one-half and one-quarter titre respectively, likewise completely absorbed the E. Ledingham agglutinins. G-NH3 agglutinated to one-quarter titre and reduced the dysentery agglutinins to one-eighth. W-NH7 and W-H7, which agglutinated only to 1/320, reduced the homologous agglutinins in the serum to fifty per cent. S-NH agglutinated at 1/320 and reduced

TABLE 7

Agglutination titres—E. coli vs. E. Ledingham serum

E. LED SERUM UNABSORBED		E. LED SERUM ABSORBED WITH E. LED	E. LED SERUM ABSORBED WITH E. COLI VS. E. COLI		E. LED SERUM ABSORBED WITH E. COLI VS. E. LED	
			Absorbing organism	Final titre	Absorbing organism	Final titre
E. Led	40,960	20				
MI-H6	40,960	20	MI-H6	20	MI-H6	20
VC-H3	40,960	20	VC-H3	20	VC-H3	20
S-H6	40,960	20	S-H6	20	S-H6	20
G-NH3	10,240	20	G-NH3	20	G-NH3	5,120
S-NH	320	20	S-NH	20	S-NH	10,240
W-NH7	320	20	W-NH7	20	W-NH7	20,480
W-H7	320	20	W-H7	20	W-H7	20,480
T-H1	40,960	20	T-H1	20	T-H1	20
A-H6	20,480	20	A-H6	20	A-H6	20
W-H12	10,240	20	W-H12	20	W-H12	20
L-H2	40,960	20	L-H2	20	L-H2	20
L-H1	5,120	20	L-H1	20	L-H1	5,120
G-H2	40,960	20	G-H2	20	G-H2	20

the agglutinins to twenty-five per cent. L-H2 agglutinated to 1/5120 and reduced the serum titre after absorption to the same figure.

Findings with monovalent anti-coli rabbit sera

A monovalent rabbit serum was prepared against each of the thirteen strains of *Escherichia coli*. These sera ranged in titre from 10,240 to 40,960.

The agglutinability of the four strains of *S. dysenteriae* was studied in these sera unabsorbed, after absorption by the homolo-

gous *E. coli*, and after absorption by the heterologous *S. dysenteriae*. Absorption of each serum by its homologous antigen removed both the homologous coli agglutinins and the heterologous dysentery agglutinins. Absorption of the sera by the individual strains of *S. dysenteriae* removed the corresponding dysentery agglutinins.

The thirteen sera fall into two groups on the basis of the behavior of the E. Ledingham dysentery bacillus. The first group of five, with the exception of one, W-NH, fail to reveal any antigenic relationship between the homologous *E. coli* and the E. Ledingham Sonne. The second group of eight sera, on the other hand, afford evidence of close relationship between the homologous antigens and the heterologous E. Ledingham.

The five sera of group one (table 8) failed to agglutinate E. Ledingham above 1/640. In one instance, W-NH, absorption of the serum with this strain of *S. dysenteriae* reduced the titre for the homologous *E. coli* fifty per cent. The other sera were not affected.

Varying reactions were obtained with the Flexner strains. The PB24 Flexner agglutinated to full titre in unabsorbed S-NH serum. Absorption of the serum with PB24 completely removed the homologous coli agglutinins. The Y Ledingham and Karim Kahn Flexners, agglutinated at one-half and one-eighth titre respectively. Each completely absorbed the homologous coli agglutinins.

W-NH serum, titre 40,960, agglutinated Karim Kahn to one-quarter titre. Absorption with this organism reduced the homologous coli agglutinins to 640. Y Ledingham, which agglutinated at one-eighth titre, did not absorb the homologous agglutinins. Conversely, E. Ledingham and PB24, agglutinating at 1/640, reduced the serum titre for the homologous coli to fifty per cent.

G-NH3 serum agglutinated PB24 at 1280. Absorption with this strain of Flexner reduced the titre for the homologous antigen to fifty per cent. Two of the other dysentery antigens agglutinated at low titre but failed to absorb.

W-H7 serum agglutinated the three Flexner strains at one-half,

one-quarter and one-eighth titre respectively. Absorption with these organisms reduced the homologous coli agglutinins to one-

TABLE 8

Agglutination titres—S. dysenteriae vs. E. coli sera

E. COLI SERUM	TITRES UNABSORBED SERUM		SERUM ABSORBED WITH S. DYSENTERIAE VS. S. DYSENTERIAE		SERUM ABSORBED WITH S. DYSENTERIAE VS. HOMOLOGOUS E. COLI	
			Absorbing organism	Final titre corresponding organism	Absorbing organism	Final titre for homologous E. coli
G-NH3	G-NH3	20,480				
	E. Led	640	E. Ledingham	20	E. Ledingham	20,480
	PB24	1,280	PB24	20	PB24	10,240
	KK	0	Karim Kahn		Karim Kahn	
	Y Led	640	Y Ledingham	20	Y Ledingham	20,480
S-NH	S-NH	10,240				
	E. Led	320	E. Ledingham	20	E. Ledingham	10,240
	PB24	10,240	PB24	20	PB24	20
	KK	1,280	Karim Kahn	20	Karim Kahn	20
	Y Led	5,120	Y Ledingham	20	Y Ledingham	20
W-H7	W-H7	40,960				
	E. Led	640	E. Ledingham	20	E. Ledingham	40,960
	PB24	5,120	PB24	20	PB24	10,240
	KK	10,240	Karim Kahn	20	Karim Kahn	5,120
	Y Led	20,480	Y Ledingham	20	Y Ledingham	20,480
W-NH	W-NH	40,960				
	E. Led	640	E. Ledingham	20	E. Ledingham	20,480
	PB24	640	PB24	20	PB24	20,480
	KK	10,240	Karim Kahn	20	Karim Kahn	640
	Y Led	5,120	Y Ledingham	20	Y Ledingham	40,960
L-H1	L-H1	40,960				
	E. Led	80	E. Ledingham	20	E. Ledingham	40,960
	PB24	160	PB24	20	PB24	40,960
	KK	5,120	Karim Kahn	20	Karim Kahn	5,120
	Y Led	160	Y Ledingham	20	Y Ledingham	40,960

half, one-quarter and one-eighth titre. Karim Kahn, which agglutinated at one-quarter titre, proved to be the most active absorber.

The L-H1 serum reacted significantly with only one of the Flexner antigens. Karim Kahn agglutinated at one-eighth titre, and when used to absorb the serum reduced the titre for the homologous *E. coli* to a like level.

The remaining eight sera reveal a close antigenic relationship between the homologous *E. coli* and the E. Ledingham (Sonne) bacillus. There is a less close relationship with the three Flexner strains (table 9). Each strain of *E. coli* was hemolytic and each produced a serum with titre of 40,960.

Six of these coli sera agglutinated E. Ledingham to full titre. Absorption of these six sera by E. Ledingham completely removed the homologous *E. coli* agglutinins. The A-H6 and L-H2 sera agglutinated E. Ledingham to one-half titre. This organism completely absorbed the homologous agglutinins from both sera.

The Flexner organisms were not strongly agglutinated by these coli anti-sera. In one instance the titre reached 5,120, in three 2,560, and in four 1,280. Despite this they exhibited some capacity to absorb the homologous coli agglutinins.

The homologous coli titre of MI-H6 serum was reduced fifty per cent after absorption with PB24. Absorption of VC-H3 serum by Y Ledingham reduced the homologous titre seventy-five per cent, even though this strain of *S. dysenteriae* agglutinated very weakly. Similarly, Karim Kahn and Y Ledingham removed seventy-five per cent of the homologous agglutinins from S-H6 serum. The T-H1 homologous titre was similarly reduced by PB24. Y Ledingham reduced the titre of G-H2 serum seventy-five per cent and PB24 fifty per cent. The A-H6 homologous titre was reduced seventy-five per cent after absorption by each of the three Flexner strains. The titre of the L-H2 serum was reduced fifty per cent by absorption with Y Ledingham. The titre of one serum, W-H12, was unaltered after absorption by the Flexner strains.

SUMMARY

The agglutination and absorption studies with patients' serum yielded evidence suggesting an immunological relationship between strains of *E. coli* and the type strains of *S. dysenteriae*.

TABLE 9

Agglutination titres—S. dysenteriae vs. E. coli sera

E. COLI SERUM	TITRES UNABSORBED SERUM		SERUM ABSORBED WITH S. DYSENTERIAE VS. S. DYSENTERIAE		SERUM ABSORBED WITH S. DYSENTERIAE VS. HOMOLOGOUS E. COLI	
			Absorbing organism	Final titre corresponding organism	Absorbing Organism	Final titre for homologous E. coli
MI-H6	MI-H6	40,960				
	E. Led	40,960	E. Ledingham	20	E. Ledingham	20
	PB24	2,560	PB24	20	PB24	20,480
	KK	320	Karim Kahn	20	Karim Kahn	40,960
	Y Led	640	Y Ledingham	20	Y Ledingham	40,960
VC-H3	VC-H3	40,960				
	E. Led	40,960	E. Ledingham	20	E. Ledingham	20
	PB24	160	PB24	20	PB24	40,960
	KK	640	Karim Kahn	20	Karim Kahn	40,960
	Y Led	320	Y Ledingham	20	Y Ledingham	10,240
S-H6	S-H6	40,960				
	E. Led	40,960	E. Ledingham	20	E. Ledingham	20
	PB24	320	PB24	20	PB24	40,960
	KK	640	Karim Kahn	20	Karim Kahn	10,240
	Y Led	640	Y Ledingham	20	Y Ledingham	10,240
T-H1	T-H1	40,960				
	E. Led	40,960	E. Ledingham	20	E. Ledingham	20
	PB24	1,280	PB24	20	PB24	10,240
	KK	5,120	Karim Kahn	20	Karim Kahn	40,960
	Y Led	1,280	Y Ledingham	20	Y Ledingham	40,960
W-H12	W-H12	40,960				
	E. Led	40,960	E. Ledingham	20	E. Ledingham	20
	PB24	160	PB24	20	PB24	40,960
	KK	1,280	Karim Kahn	20	Karim Kahn	40,960
	Y Led	640	Y Ledingham	20	Y Ledingham	40,960
G-H2	G-H2	40,960				
	E. Led	40,960	E. Ledingham	20	E. Ledingham	20
	PB24	640	PB24	20	PB24	20,480
	KK	640	Karim Kahn	20	Karim Kahn	40,960
	Y Led	320	Y Ledingham	20	Y Ledingham	10,240
A-H6	A-H6	40,960				
	E. Led	20,480	E. Ledingham	20	E. Ledingham	20
	PB24	1,280	PB24	20	PB24	10,240
	KK	2,560	Karim Kahn	20	Karim Kahn	10,240
	Y Led	2,560	Y Ledingham	20	Y Ledingham	10,240
L-H2	L-H2	40,960				
	E. Led	20,480	E. Ledingham	20	E. Ledingham	20
	PB24	320	PB24	20	PB24	40,960
	KK	640	Karim Kahn	20	Karim Kahn	40,960
	Y Led	640	Y Ledingham	20	Y Ledingham	20,480

Absorption of nine of these sera by the corresponding strain of *E. coli*, in each instance, completely removed agglutinins for the latter and for one or more of the *S. dysenteriae* strains as well. Conversely, in the five sera studied, absorption by *S. dysenteriae* removed both the dysentery and the coli agglutinins. Since it was impossible to titrate the absorbing doses due to insufficient serum, over-absorption may have been a factor in determining these results. The frequency with which agglutinins for only one of the antigens was affected renders this improbable.

Additional evidence of antigenic relationship was obtained from the studies using monovalent dysentery anti-sera. All thirteen strains of *E. coli* agglutinated to varying titre in these sera. Absorption by the homologous antigen removed both the homologous dysentery and the heterologous coli agglutinins. Absorption with the individual strains of *E. coli* removed the corresponding coli agglutinins and reduced the homologous titre of the sera in varying degree.

Seven of the strains of *E. coli*, agglutinating in the unabsorbed PB24 serum, reduced the homologous titre after absorption by fifty per cent or more. This points to an antigenic component common to these strains of *E. coli* and the PB24 *S. dysenteriae*. The six strains which were agglutinated by the PB24 serum but failed to absorb the homologous agglutinins lack this common factor. Their agglutination, therefore, must be regarded as non-specific.

Eight of the coli strains agglutinated in unabsorbed Karim Kahn serum, and, when used to absorb the serum, reduced the homologous titre by fifty per cent or more. Five strains failed to absorb the homologous dysentery agglutinins and must, therefore, be considered antigenically unrelated to the Karim Kahn Flexner bacillus. Agglutination of these organisms by this serum must be classed as non-specific.

Nine of the *E. coli* strains, agglutinating in the unabsorbed Y Ledingham serum, when used to absorb this serum reduced the homologous titre by fifty per cent or more. Four failed to absorb the dysentery agglutinins and are therefore unrelated to the Y Ledingham Flexner bacillus. Agglutination of these organisms in this serum is consequently non-specific.

The results obtained with the E. Ledingham (Sonne) serum were striking. Six of the strains of *E. coli* agglutinated at full titre and completely absorbed the dysentery agglutinins. Two other strains, agglutinating at lower dilutions, likewise completely absorbed the homologous agglutinins. The remaining five strains, although agglutinating only in low dilutions, reduced the homologous serum titre by fifty per cent or more. These observations, therefore, point to agglutinogenic identity of six strains of *E. coli* and the E. Ledingham (Sonne) bacillus. The remaining seven strains appear to be closely related.

Confirmatory data were obtained from the studies of the thirteen monovalent anti-coli sera. The strains of *E. coli* fall into two groups, one unrelated to, and the second closely related to, if not antigenically identical with, the E. Ledingham (Sonne) bacillus.

The first group comprises five sera, none of which agglutinated E. Ledingham in high dilutions. Absorption of these sera with E. Ledingham in only one instance reduced the homologous coli titre by fifty per cent. The Flexner strains gave varying reactions. PB24 agglutinated to the full titre of the S-NH serum and completely removed the homologous coli agglutinins. The remaining four coli sera agglutinated the Flexner strains in lower dilutions. Absorption with one or more of these strains reduced the homologous titre of each serum by fifty per cent or more. These observations indicate an antigenic relationship, identical in one instance and close in a second, between the Flexner strains of *S. dysenteriae* and the five strains of *E. coli* comprising Group 1. It is interesting to note that non-specific agglutination of a Flexner bacillus may reach one-eighth of the titre of a monovalent coli anti-serum.

The behavior of the *S. dysenteriae* antigens in the eight monovalent coli sera comprising Group 2 points to agglutinogenic identity of the E. Ledingham (Sonne), and six of the strains of *E. coli*, and to a close relationship with two of the others. Despite the low titre agglutinations of the Flexner strains in these sera, the agglutinin absorption reactions noted indicate a serologic relationship between our Flexner antigens and seven of the strains of *E. coli* which may be of significance.

Comparison of the agglutination and agglutinin absorption activity of the four strains of *S. dysenteriae* and the thirteen strains of *E. coli* in the monovalent dysentery antisera and in the monovalent coli antisera reveals significant findings (table 10). These are not in exact conformity with the data obtained by the sera singly. Reciprocal agglutination and reciprocal absorption in the dysentery and the coli antisera demonstrate antigenic identity between E. Ledingham (Sonne) and five strains of *E. coli*. There is a close relationship with four additional coli strains.

TABLE 10

Evidence of relationship between E. coli and S. dysenteriae from reciprocal agglutination and reciprocal absorption experiments

E. COLI	S. DYSENTERIAE, PB 24		S. DYSENTERIAE, KARIM KAHN		S. DYSENTERIAE, Y LEDINGHAM		S. DYSENTERIAE, E. LEDINGHAM	
	Dysentery antiserum	Coli antiserum	Dysentery antiserum	Coli antiserum	Dysentery antiserum	Coli antiserum	Dysentery antiserum	Coli antiserum
MI-H6	0	+	0	0	+	0	+	+
VC-H3	0	0	0	0	0	+	+	+
S-H6	0	0	+	+	+	+	+	+
G-NH3	+	+	0	0	+	0	+	0
S-NH	+	+	+	+	+	+	+	0
W-NH7	0	+	+	+	+	0	+	+
W-H7	+	+	+	+	+	+	+	0
T-H1	+	+	+	0	0	0	+	+
A-H6	+	+	+	+	+	+	+	+
W-H12	0	0	0	0	0	0	+	+
L-H2	+	0	0	0	+	+	+	+
L-H1	0	0	+	+	0	0	+	0
G-H2	+	+	+	0	+	+	+	+

No such evidence of identity was found between the Flexner strains and the *E. coli*. Partial agglutination in the sera and significant reduction of homologous agglutinin titres after absorption was observed. Similar findings in both the coli and the dysentery antisera indicate a serologic relationship between six strains of *E. coli* and PB24 Flexner. Six coli strains likewise appear to be related to Karim Kahn Flexner, and six to Y Ledingham Flexner.

Five of the strains of *E. coli* are related to only one of the *S. dysenteriae* strains. Three of the coli are related to two of the strains of *S. dysenteriae*, four are related to three of the *S. dysenteriae* strains, and one, A-H6, is related to all.

DISCUSSION

Four possible explanations may be advanced to account for these immunological cross-relationships between *Shigella dysenteriae* and *Escherichia coli*. They may represent a major and minor agglutinin phenomenon dependent upon non-specific group agglutinins. They may be regarded as an instance of paragglutination. Unrecognized "R" dissociation of the cultures may have occurred with resultant development of common rough somatic antigens. A true antigenic relationship may exist.

Complete removal of both homologous and heterologous agglutinins by absorption of the monovalent rabbit sera with their homologous antigens suggests a simple major and minor agglutinin phenomenon. Heterologous absorption, however, presents strong evidence of a fundamental agglutinogenic similarity. Moreover, the use of living antigens to absorb the sera insures maximal removal of both major and minor agglutinins. The use of formolized antigens for the agglutination reactions minimizes non-specific group agglutination.

The phenomenon of paragglutination is characterized by the agglutination of organisms other than the known etiologic agent in the serum of an infected individual. The Weil-Felix reaction in typhus fever is a classic example. As previously noted, various workers have observed that certain strains of *Escherichia coli* recovered from dysentery patients may be agglutinated in monovalent dysentery anti-sera. Two explanations of this phenomenon have been advanced. It is postulated that close association of *Escherichia coli* and *Shigella dysenteriae* within the patients intestinal tract alters the receptor apparatus of the *Escherichia coli* to resemble that of the *Shigella dysenteriae*. Arkwright (1931), however, considers that paragglutination is due to the presence of similar or identical "R" antigens.

The agglutinogenic cross-relationships which we have ob-

served might be classed as instances of paragglutination. This seems unlikely, if that phenomenon, in fact, is dependent upon alteration of receptor apparatus. The strains of *Escherichia coli* which we have examined were obtained from patients who never afforded cultural evidence of infection by *Shigella dysenteriae* despite repeated examination.

There remains the possibility that similar or identical "R" antigens may be present in the strains which we have been investigating. It is recognized that colony inspection and failure to flocculate in 0.85 per cent sodium chloride solution do not constitute positive proof of a pure "S" phase. The possibility of common "R" agglutinins has been avoided as carefully as present methods permit. None the less, partial "R" dissociation may have occurred and may account for the cross-relationships observed.

The general uniformity of results obtained by reciprocal agglutination and reciprocal absorption in the human sera, in the monovalent dysentery anti-sera, and in the monovalent coli antisera are strongly suggestive of a fundamental antigenic relationship between strains of *S. dysenteriae* and *E. coli*. Agglutination to full titre of a known monovalent anti-serum, and complete absorption of the homologous agglutinins of such a serum are commonly accepted as proof of antigenic identity of an unknown organism and the antigen used to produce the serum. Recognition of less close degrees of relationship is based upon partial agglutination and less complete absorption of homologous agglutinins. Judged by these criteria, subject to the potential error of unrecognized partial "R" dissociation, a true antigenic relationship exists between the four strains of *Shigella dysenteriae* and the thirteen strains of *Escherichia coli* studied.

The coli strains do not appear to be identical. Eight strains were non-motile. Ten were hemolytic on rabbit blood agar. Their respective immunological relationships will be reported subsequently.

We have previously called attention to the necessity for caution in the interpretation of the agglutination reaction for *S. dysenteriae* in chronic inflammatory disease of the colon. The

studies here presented give added emphasis. They demonstrate that strains of *Escherichia coli* recovered from such patients may give rise to relatively high titre agglutinins for strains of *S. dysenteriae* Sonne and Flexner. This leads inescapably to the conclusion that a positive agglutination reaction with these organisms does not constitute proof of homologous infection. Certain diagnosis of bacillary dysentery, therefore, rests upon, and only upon, recovery of *S. dysenteriae* on culture.

CONCLUSIONS

1. A close agglutinogenic relationship exists between certain strains of *Escherichia coli* and *Shigella dysenteriae* Sonne and Flexner.

2. The relationship between the strains of *Escherichia coli* and *Shigella dysenteriae* Sonne is closer than that between *Escherichia coli* and *Shigella dysenteriae* Flexner.

3. This relationship is such as to produce high titre heterologous agglutinins in the experimental animal.

4. These observations demonstrate that the agglutination reaction, unsupported by confirmatory bacteriologic evidence, does not constitute valid proof of infection by *Shigella dysenteriae* Sonne and Flexner.

5. The diagnosis of bacillary dysentery can be substantiated only by recovery of the organism.

REFERENCES

ARKWRIGHT, J. A. 1931 Agglutination in a system of bacteriology, **6**, 381–423. H. M. Stationery Office, London.

BULLOCH, W. 1929 The colon group and similar bacteria. A system of bacteriology, **4**, 274. H. M. Stationery Office, London.

DUVAL, C. W. AND SHORER, E. H. 1904 Studies of the diarrhoeal diseases of infancy. Studies from The Rockefeller Inst. Med. Res., **2**, 43–54.

GAY, F. P. 1935 Agents of disease and host resistance. Charles C. Thomas, Springfield, Ill. Baltimore, Md.

HEGEDUS, A. 1933 A Paragglutinatios Coli Torzsekrol es Azok diagnostikai ertekeleserol. Magyar Orvosi Archivum, **34**, 287–297.

KLIGLER, I. F. 1918 A study of the antiseptic properties of certain organic compounds. J. Exp. Med., **27**, 463–478.

KUHN, P., GILDEMEISTER, E. AND WAITHE. 1911 Ueber bakteriologische Beobachtungen bei Irren-Ruhr unbesondere über die Erscheinung der Paragglutination. Arb. a. k. k. Gesundhtsamte, **31**, 394–430.

MACKIE, T. T. The specificity of the agglutinin reaction for *Shigella dysenteriae*. I. The agglutination reaction in chronic bacillary dysentery. A serologic and bacteriologic study of 47 cases. Arch. Int. Med., in press.

MURRAY, E. G. D. 1918 An attempt at classification of *Bacillus dysenteriae* based upon an examination of the agglutinating properties of fifty-three strains. J. Royal Army Med. Corps, Oct.–Nov.

PARK, W. H. AND WILLIAMS, A. H. 1910 Pathogenic microörganisms. Lea and Febiger, New York and Philadelphia.

SCHÜTZE, H. 1921 The permanence of serological paratyphoid B types with observations on the non-specificity of agglutination with "rough" variants. J. Hygiene, **20**, 330–341.

THIBAULT, P. AND BRAUNBERGER, J. 1935 Antigenic variations S and R of the Shiga bacillus. Compt. rend. soc. de biol., **120**, 617–618.

WHITE, P. B. 1926 Further studies of the Salmonella group. Privy Council, Med. Res. Committee, Sp. Report Series, No. 103. H. M. Stationery Office, London.

WHITE, P. B. 1929 Notes on intestinal bacilli with special reference to smooth and rough races. J. Path. and Bact., **32**, 85–94.

WINKELSTEIN, A. Personal communication.

ZINSSER, H. AND BAYNE-JONES, S. 1935 A text book of bacteriology. D. Appleton-Century Co., 7th ed., New York, N. Y.

THE IMPLANTATION OF ORAL AND INTESTINAL STRAINS OF L. ACIDOPHILUS IN THE ALBINO RAT[1]

MICHAEL J. PELCZAR, JR.,[2] AND LUTHER A. BLACK

Department of Bacteriology, University of Maryland, College Park

Received for publication June 30, 1938

During the past few years some investigators have ascribed to lactobacilli a definite rôle in the production of dental caries. A few have concluded from their studies that these lactobacilli were *Lactobacillus acidophilus* species, identical with those of intestinal origin, for example, Bunting (1937) states: ". . . dental caries is a specific bacterial disease and the specific organism involved is *L. acidophilus*." The principal exponents of these views include Howe and Hatch (1917), Rodriguez (1922), Bunting and Palmerlee (1925), Rosebury, Linton, and Buchbinder (1929), Howitt and van Meter (1930), Hadley (1933), Johnston, Williams, Anderson, Tisadall and Kaake (1936), Bunting (1937), and Jay (1937).

Other investigators are diametrically opposed to the foregoing claims as evidenced by many publications. Morishita (1929), Rettger (1932), Weinstein, Anderson and Rettger (1933), and Rettger, Levy, Weinstein and Weiss (1935), have concluded from their studies that the oral and intestinal strains of lactobacilli are distinctly separate groups of organisms, and they have not been able to demonstrate a striking correlation between the occurrence of caries and the presence of *L. acidophilus*.

In view of these contradictory opinions and since reported

[1] Based on a portion of the thesis submitted by the senior author to the Graduate School of the University of Maryland in partial fulfillment of the requirements for the degree of Master of Science.

[2] Present address, Department of Bacteriology, State University of Iowa, Iowa City.

results indicate that cultural, morphological, biochemical and serological characteristics afford a poor basis for differentiation of these lactobacilli, the present investigation was undertaken to determine whether the organisms of either oral or intestinal origin differed in their ability to become implanted in the intestinal tract of white rats.

EXPERIMENTAL

Methods

Cultures—type and source. A. *Oral or dental strains:* Nos. 14, 18, and 19 were obtained from L. F. Rettger, labelled S_s, Flynn, and Rosebury respectively. Nos. 60, 61, 94, and 97 were obtained from H. R. Curran.[3] All of these cultures were of the smooth (Y) type. B. *Intestinal strains:* No. 64 was obtained from Curran, originally isolated from acidophilus milk, nos. 15 and 20 from Rettger,[4] and nos. 1 and 9 from R. P. Meyers. All of these were rough (X), filamentous colony types. Nos. 10 and 12 were received from J. M. Sherman, K from C. W. England, and K4y from N. Kopeloff. These were smooth (Y) intestinal types. C. *Lactobacillus bulgaricus:* Only one culture was used. This was obtained from Sherman, originally from Rettger.

Preparation of milk culture. The milk culture used for feeding was prepared by the inoculation of skimmed milk, sterilized by autoclaving. Cultures used for inoculum in the preparation of the fermented milk and for daily feeding were forty-eight hours old. Plate counts were made of each fermented milk several times during the course of this work and with but two exceptions these indicated that the viable organisms in each preparation were several hundred million per cc.

Animals and diet. The implantation of the organisms was determined by feeding experiments conducted with male and female albino rats. Before use in the experiments the rats were maintained on a ration of commercial dog pellets which kept them

[3] Originally from Enright, his nos. 144 and 42, the Morishita collection no. 13, and Kulp, his S b, respectively.

[4] The Wickerham and Cohen starter strains respectively.

in an apparently healthy condition. Two weeks previous to the administration of a culture, each rat was placed in a separate cage and fed solely upon 10 grams of ground beef daily in addition to water. At the end of the two-week period, the intestinal flora, as determined by microscopic examination of gram-stained smears and plate cultures from fecal specimens, was composed almost entirely of gram-negative bacteria. After this period, the rats were fed approximately 5 cc. of the lactobacilli preparations daily (one strain for each rat), in addition to the ground beef. As controls one rat was fed meat only, and another rat 5 cc. of sterile skimmed milk daily in addition to meat. The feeding continued for three weeks, after which five of seventeen rats were sacrificed for bacteriological examination of intestinal contents (table 2). The remaining rats were then fed a diet of meat (10 grams), sterile skimmed milk (5 cc.), and lactose (1 gram) for two weeks during which time fecal examinations were made.

Collection of feces and intestinal contents. Fecal specimens were collected regularly at three-day intervals while feeding cultures, and after 3, 6 and 14 days during lactose feeding. Specimens were collected as nearly as possible under aseptic conditions into sterile petri dishes.

The entire intestinal tract was removed from each rat sacrificed, sections selected from the duodenum, ileum, caecum, and colon, and the contents ejected into a sterile 9 cc. physiological saline dilution blank.

Bacteriological examination. A portion of each fecal specimen, approximately one-fourth of a gram, was disintegrated into a uniform suspension in 9 cc. of sterile physiological saline. The collection of intestinal specimens in 9 cc. blanks sufficed for the initial dilution. Smears were made from these original suspensions and gram-stained, and further dilutions of 1:1,000, 1:10,000, and 1:1,000,000 were prepared for plating. While the dilutions were not exactly quantitative (since the original sample was approximate) this did not interfere with the results inasmuch as determination of the number of bacteria per gram of feces was not attempted. Identification of *L. acidophilus*, and determination of its approximate percentage of the total bacterial flora were the primary objectives.

The plating medium used was that suggested by Rettger *et al.* (1935) composed of tomato juice, peptonized milk, neopeptone, and yeast extract. All plates were incubated in an atmosphere of 10 per cent carbon dioxide at 37°C. for 3–5 days. Following incubation, the plates containing the most suitable number of colonies for examination were selected for further study. By means of a dissecting microscope the various colony types were examined and their description recorded, together with the approximate percentage of each type. Gram stains were made of each colony type, and tubes of litmus milk were inoculated from colonies resembling those of lactobacilli. Litmus milk tubes showing reactions characteristic of the lactobacilli were held for identification studies. Sometimes it required several transfers before a typical reaction occurred. Tubes in which curdling and reduction of litmus milk did not occur were discarded.

Identification of cultures. It was not our aim to make a detailed study of the cultural and biochemical characteristics of all strains employed. Our primary objective was to determine a few characteristics of each strain before implantation and then repeat these same tests upon *acidophilus-like* organisms which were isolated from the feces. In this manner we could determine whether the recovered organism was the same one which was being administered orally. For this purpose the morphology, colonial characteristics, ability to ferment maltose, mannitol, sucrose and raffinose, and the phenol resistance of each original strain, as well as of all isolated strains, were recorded. Lipolytic activity, although not determined in this study, has been suggested for differentiation of closely related strains of lactobacilli, and Sabine (1937) indicated that this was valuable in clinical work to demonstrate that recovered strains were identical to administered strains.

Fermentation studies were carried out in precisely the same manner described by Curran, Rogers, and Whittier (1933) except that a proteose-peptone yeast-extract broth was used as a basic medium instead of casein-digest broth. In preliminary experiments this proteose-peptone yeast-extract broth was shown capable of supporting good growth of both oral and intestinal strains of

lactobacilli. Its use was advantageous, both in simplicity of preparation and in uniformity of composition of different batches of medium, which may be variable with casein-digest broth. Phenol tolerance was determined as described by Kulp (1929) and employed by Curran, Rogers and Whittier, except that agar shake cultures were substituted for plate cultures.

Results

An example of the detailed manner in which all fecal specimens were examined is given in table 1, such results being recorded for each rat used. Although the examination of the stained smears of fecal specimens along with plating and macroscopic and microscopic examination of resulting colonies were useful in following the change in fecal flora, we were interested primarily only in identification of lactobacilli strains. Table 1 also illustrates the detailed plan used in partially identifying lactobacilli recovered after implantation, some additional characteristics used for this purpose being listed in table 3. This is mentioned, particularly, since it is our opinion that the process of identifying lactobacilli in some earlier studies of implantation may have been done hastily and perhaps inadequately.

A summary of the percentages of lactobacilli present in each specimen examined by the above-mentioned procedure is given in table 2. It will be noted that two figures are given for each analysis, the first indicating the *entire Lactobacillus* content of the feces irrespective of type, while the second percentage refers to the *type administered* to that particular animal, as determined by certain characteristics apparently of value in differentiation as summarized in table 3.

Controls. The rat (no. 2) subsisting on the basic diet (10 grams of ground beef) had a fecal flora in which gram-negative organisms predominated. Lactobacilli could not be isolated by cultural methods. Rat 1 which received 5 cc. of sterile skimmed milk in addition to ground beef yielded lactobacilli in the feces after two weeks, and later, when the animal received lactose, these organisms were predominant, almost to the exclusion of all other types.

Oral strains. Rats fed oral strains of lactobacilli and the one

TABLE 1

Typical results of microscopic and cultural examination of fecal specimens from a white rat receiving L. acidophilus culture

Rat 13. Culture 1 (intestinal (X) strain). Diet 10 grams beef + 5 cc. of milk culture

DAYS ON DIET	FECAL SUSPENSION		COLONIES		ORGANISMS		L. A.
	Stain, shape	Description	Description	Per cent	Stain, Shape	Description	
							per cent
3	Gram + rod	Long-single, pairs	Subsurface filamentous	80	Gram + rod	Long, thin-single, short chains	80
	+ rod	Short-single, chains	Subsurface				
	− rod	Short, single	Elliptical smooth	15	+ rod	Short, single-pairs, chains	
	+ cocci	Single and pairs	Surface circular, large	5	− rod	Short, single	
6	+ rod	Long-single, pairs, chains	Subsurface, very filamentous	95	+ rod	Long, thin-single, short and long chains	95
	+ rod	Short-single, pairs, chains					
	+ cocci	Single, pairs (large)	Subsurface, circular and elliptical	5	+ rod	Short-single, chains	
	− rod	Single, long and short					
9	+ rod	Long-single, chains	Subsurface, extremely filamentous	99	+ rod	Long, thin-single, chains	99
	+ rod	Short-single, chains					
	+ cocci	Single-pairs, chains	Subsurface, circular and elliptical	1	+ rod	Short-single, chains	
12	+ rod	Long, thin-single chains	Subsurface, extremely filamentous	99	+ rod	Long, thin-single, chains	99
	+ rod	Short-single, chains					
	+ cocci	Single and pairs	Subsurface, circular and elliptical	1	+ rod	Short, single-pairs, chains	
	− rod	Single, short					
15	+ rod	Long, thin-single, chains	Subsurface, extremely filamentous	90	+ rod	Long, thin, single and chains	90
	+ rod	Short-single, chains					
	+ cocci	Single, pairs	Subsurface, circular and elliptical	10	+ rod	Short-single, chains	
	− rod	Short, single					

	− rod	Short, single					
18	+ rod	Long, thin-single, pairs, chains	Subsurface, extremely filamentous	99	+ rod	Long, thin-single, chains	99
	+ rod	Short-single, chains					
	+ cocci	Single, pairs (large)	Subsurface, circular, elliptical	1	+ rod	Short-single, chains	
	− rod	Short-single					
21*	+ rod	Long, thin-single, pairs, chains	Subsurface, extremely filamentous	99	+ rod	Long, thin-single, chains	99
	+ rod	Short-single, chains	Subsurface, circular and elliptical	1	+ rod	Short-single, chains	
	+ cocci	Single, pairs					
24	+ rod	Long, thin-single, short chains	Subsurface filamentous	80	+ rod	Long, thin-single, chains	100
	+ rod	Short-single, short chains	Surface and subsurface circular, elliptical	20	+ rod	Short-single, pairs	
	+ cocci	Large-single, pairs					
27	+ rod	Short-single and short chains	Surface and subsurface circular, elliptical	50	+ rod	Short-single, short chains	
	+ rod	Long, thin-single, short chains		45	+ rod	Long, thin-single, chains	45
	+ cocci	Large-single, pairs, short-single (very few)	Subsurface filamentous; surface, circular, large	5	− rod	Short-single	
35	+ rod	Short-single, short chain	Surface and subsurface circular and elliptical	80	+ rod	Short-single, pairs and short chains	
	+ rod	Long, thin-single, pairs		10	− rod	Short-single	
	+ cocci	Large-single, pairs	Surface, circular, moist; subsurface filamentous	10	+ rod	Long thin-single and pairs	10
	− rod	Short-single					

L. A. Percentage of *L. acidophilus* of the strain present in the milk culture administered.

* Last day of administration of milk culture—*L. acidophilus* feeding replaced by 5 cc. of sterile skimmed milk and one gram of lactose.

BOOK B

TIGHT

BOUND

HTLY

TABLE 2

Percentage of Lactobacilli in the feces of albino rats and the percentage of the total comprised of organisms administered orally in milk cultures

DIET: GROUND BEEF PLUS	RAT NO.	DAYS ON DIET									
		3	6	9	12	15	18	21*	24	27	35
		(Per cent Lactobacilli as determined from tomato agar plates)									
		A/A′	A/A′	A/A′	A/A′	A/A′	A/A′	A/A′	A/A′	A/A′	A/A′
Controls:	2	0/0	0/0	0/0	0/0	0/0	0/0	0/0†			
Sterile milk	1	0/0	0/0	0/0	0/0	40/0	40/0	40/0	80/0	80/0	90/0
Dental strains:											
14	8	100/0	85/0	90/0	95/0	95/0	85/0	90/0†			
18	9	0/0	80/0	90/0	90/0	90/0	90/0	80/0	90/0	90/0	90/0
19	10	0/0	40/0	90/0	95/0	95/0	95/0	95/0†			
60	6	0/0	20/0	90/0	80/0	90/0	90/0	90/0	90/0	80/0	90/0
61	3	0/0	20/0	90/0	90/0	90/0	90/0	85/0	70/0	80/0	90/0
94	7	0/0	90/0	90/0	100/0	100/0	100/0	100/0†			
97	4	80/0	90/0	80/0	80/0	90/0	90/0	80/0	80/0	80/0	90/0
L. bulgaricus:											
B	5	10/0	80/0	85/0	80/0	85/0	90/0	90/0†			
Intestinal strains (rough):											
1	13	95/80	100/95	100/99	100/99	100/90	100/99	100/99	100/80	95/45	90/10
9	19	60/10	95/25	100/60	100/80	100/80	100/80	100/80	100/25	95/40	70/30
15	18	75/25	100/95	100/98	100/100	100/100	100/100	100/100	100/50	100/45	65/5
20	15	90/30	100/95	100/90	100/90	100/100	100/100	100/100	100/80	100/25	90/15
64	12	95/0	95/0	100/0	100/0	100/0	100/0	100/0	100/0	90/0	90/0
Intestinal strains (smooth):											
10	16	70/20	70/20	90/30	100/40	100/70	100/90	100/90	100/60	80/30	70/0
12	14	30/0	90/40	95/35	100/40	100/55	100/60	100/50	80/20	50/0	60/0
K	17	90/30	95/45	95/35	95/35	95/45	95/35	95/35	100/20	95/5	80/0
K4y	11	60/30	60/30	90/70	90/80	100/90	95/85	100/80	80/30	75/5	90/0

* Last day of administration of milk culture—diet changed to sterile skimmed milk (5 cc.) and ground beef (10 grams), plus lactose (1 gram).

A—Percentage of total fecal flora composed of *lactobacilli*.

A′—Percentage of total fecal flora present representing the *strain administered* in the milk culture.

† Sacrificed for bacteriological examination of intestinal contents.

rat receiving a culture of *L. bulgaricus* exhibited the same characteristic trend with respect to intestinal transformation. In each case, without exception, lactobacilli predominated after the first few days of feeding. However, the lactobacilli which were isolated from these rats differed slightly in their colonial characteristics from the strains which were being fed. In additions, the same organism appeared in the feces of the rat receiving *L. bulgaricus* and a control rat receiving sterile skimmed milk. This made it evident that the recovered organisms were not the oral strains being fed to the rats, but instead, some other lactobacilli, apparently a rat strain initially present in the intestinal tract and stimulated by milk in the diet. As will be shown later, by comparing the cultural and physiological characteristics of the organisms fed and isolated, we were able to conclude that the oral strains were not present in the feces. Similarly *L. bulgaricus*, was never recovered from the feces of the rat receiving this culture. At the end of three weeks, when lactobacilli culture feeding ceased, and the diet of five rats of this group consisted of ground beef, sterile skimmed milk, and one gram of lactose, there was practically no change in the intestinal flora of those rats which had previously received lactobacilli cultures. The control rat (no. 1) which had been fed meat and sterile skimmed milk, and now received lactose in addition, yielded a marked increase of lactobacilli.

The remaining five rats of this group, the one fed ground beef, the one fed *L. bulgaricus*, and three which had received oral strains of lactobacilli were sacrificed. Upon examination of specimens from the duodenum, ileum, caecum and colon of each animal, organisms of the original strains could not be isolated although lactobacilli, apparently rat strains and identical with those found in previous examinations of the feces were recovered. With the exception of duodenal specimens where organisms were relatively scarce and in one case absent, bacteriological examinations of sections of the intestinal tract of sacrificed animals yielded the same results as those reported from examinations of the feces of live animals.

Intestinal strains. With one exception (culture 64) the rough

A—Percentage of total fecal flora present representing the *strain administered* in the milk culture.
† Sacrificed for bacteriological examination of intestinal contents.

intestinal organisms were recovered from the corresponding rats to which they were fed. Apart from the one exception, the rough strains appeared in feces in great numbers after the first few days of feeding almost to the exclusion of all other bacteria. The smooth strains did not show such predominance, although in most cases after two weeks of feeding they constituted the majority of the bacteria present. Lactobacilli of the type isolated from rats receiving oral strains were also isolated. As can be seen from table 2, the intestinal flora of each rat in the group receiving intestinal strains of *L. acidophilus* was comprised almost entirely of lactobacilli, including both the administered intestinal strain and the rat strain apparently initially present in the intestinal tract. When *L. acidophilus* culture feeding was replaced by lactose and sterile skimmed milk there was a gradual disappearance of the strain which had been administered, with a gradual increase of the rat intestinal type. After two weeks without culture feeding the smooth intestinal strains which had been fed were no longer present in the feces, while the rough strains, with the exception mentioned previously (culture 64), were present to a very small extent. In each instance after cessation of culture feeding the rat intestinal type of lactobacilli eventually predominated over the strains fed.

Cultural characteristics. The morphology of all organisms varied considerably, which is characteristic of the *Lactobacillus* genus. Some of the rods were extremely short having the appearance of ovoid cells while others were slender and long, appearing single, in pairs and in chains. The rat strains of lactobacilli isolated were nearly always short to medium sized rods. Colonies of smooth intestinal and smooth oral types could not be differentiated. The rat strains also produced colonies which were smooth, but more dense, white, smaller, and always had an entire edge, while colonies of the rough intestinal strains were extremely filamentous. All strains reduced and curdled litmus milk after 24 or 48 hours. All strains of the oral and intestinal groups fermented maltose, sucrose (with one exception) and mannitol (with two exceptions). These exceptions occurred among the oral strains. The reaction on raffinose was variable. Those

TABLE 3

Certain cultural and biochemical characteristics of strains of Lactobacilli fed and strains isolated from the feces of albino rats

CULTURE NUMBER	RAT NUMBER	ORIGINAL CULTURES FED						CULTURES ISOLATED					
		Colony type	Fermentation of: Sucrose	Maltose	Mannitol	Raffinose	Phenol tolerance	Colony type	Fermentation of: Sucrose	Maltose	Mannitol	Raffinose	Phenol tolerance
Controls	2	(Basic diet)						(No lactobacilli isolated)					
	1	(Basic diet plus skimmed milk)						S	+	−	+	−	1:400
L. bulgaricus B	5	R	+	−	−	−	1:300	S	+	+	+	−	1:200
Oral strains (L. acidophilus):													
14	8	S	+	+	+	+	1:500	S	+	−	+	−	1:300
18	9	S	+	+	+	+	1:600	S	+	−	+	−	1:400
19	10	S	+	+	+	+	1:400	S	+	−	+	−	1:400
60	6	S	+	+	+	−	1:500	S	+	−	+	−	1:200
61	3	S	+	+	+	+	1:500	S	+	+	+	−	1:200
94	7	S	−	+	−	−	1:600	S	+	−	+	−	1:500
97	4	S	+	+	−	−	1:400	S	+	−	+	+	1:300
Intestinal strains (L. acidophilus):													
1	13	R	+	+	+	+	1:200	R*	+	+	+	+	1:200
								S	+	−	+	+	1:500
9	19	R	+	+	+	−	1:400	R*	+	+	+	−	1:300
								S	+	−	+	−	1:600
15	18	R	+	+	+	+	1:300	R*	+	+	+	+	1:300
								S	−	−	+	+	1:400
20	15	R	+	+	+	+	1:200	R*	+	+	+	+	1:200
								S	+	−	+	−	1:400
64	12	R	+	+	+	−	1:400	S	+	−	+	−	1:400
10	16	S	+	+	+	−	1:200	S*	+	+	+	−	1:200
								S	+	−	+	+	1:300
12	14	S	+	+	+	+	1:300	S*	+	+	+	+	1:200
								S	+	−	+	−	1:500
K	17	S	+	+	+	+	1:200	S*	+	+	+	+	1:200
								S	+	−	+	−	1:200
K4y	11	S	+	+	+	+	1:200	S*	+	+	+	+	1:200
								S	+	−	+	−	1:400

* Has the same characteristics as the culture originally fed, indicating implantation of the original strain.

strains isolated which were of rat origin fermented sucrose and mannitol with only one exception while the reaction on maltose and raffinose was variable. Intestinal strains were much more tolerant to phenol than the oral strains, being able to initiate growth in concentrations of 1:400 or greater. Strains of rat origin, although not as resistant to phenol as the stock intestinal strains, were slightly more tolerant than the oral strains used.

These cultural characteristics are presented in detail in table 3, wherein the characteristics of the strain administered orally is compared to those of *acidophilus-like* organisms found in the feces of the same rat. In this way it could be ascertained whether the organism isolated from the feces of a particular rat was the same one which had been fed to that rat.

As already shown, fermentation reactions alone were inadequate for classification of the strains but they were of real value, along with cultural and other characters, in determining the characteristics of a known strain before implantation. By repetition of these tests upon organisms isolated after culture feeding we could determine with considerable certainty whether or not the recovered organisms were identical with those administered. Colonies of the rat strains (always smooth) did differ in size, color and contour from the smooth human types and we believe the constancy of these characteristics and the appearance of the organisms in control rats, along with the fermentation reactions described above, warrant designation of these lactobacilli as rat strains.

DISCUSSION

The implantation of *L. acidophilus* in the intestinal tract of man and rats has been repeatedly demonstrated. Since results of the present investigation show that none of the oral strains used were implantable it seems that some differences must exist between the lactobacilli of oral and intestinal origin. This is in accord with the results of Rettger and associates, Curran, Rogers and Whittier, and Ulicny, who have reported differences between these two groups with respect to cultural characteristics, type of lactic acid produced, and the quantitative utilization

of lactose, respectively, but different from the conclusions of those who maintain that both groups are comprised of the same organisms. Considering the many similarities which exist between the oral and intestinal lactobacilli and the fact that many of the differences are quantitative in nature, we would be inclined to class the oral strains as atypical species and not the true central type, *L. acidophilus*. While no studies were made of these organisms from the standpoint of the etiology of dental caries, we are inclined to be of the same opinion as Rettger, Levy, Weinstein, and Weiss who maintain that there is still a lack of sufficient evidence to show that the intestinal types are of significance in dental caries.

The failure to implant one of the intestinal strains (64) is significant but not necessarily surprising. Except for the inability of this organism to become established in the intestinal tract of rats, its characteristics conformed to those of other strains of *L. acidophilus* which were implanted. This is significant in view of the similarity between the two species, *L. acidophilus* and *L. bulgaricus*, the latter considered not implantable. Although some have separated these species on the basis of maltose fermentation, the recent work of Curran and associates hardly substantiates such a separation. Recently Kopeloff and Kopeloff (1937) reported that R forms of both *L. acidophilus* and *L. bulgaricus* produce inactive lactic acid, while the smooth types of both produce dextro-rotary acid. Admitting the intimate relationship between these organisms, it is questionable whether our culture no. 64 should be considered an atypical strain of *L. acidophilus* or whether it belongs to the *L. bulgaricus* species. Perhaps organisms described as *L. bulgaricus* may be in reality unimplantable *L. acidophilus*. We are of the opinion that culture no. 64 is an atypical strain of *L. acidophilus* inasmuch as with other bacteria it is not unusual to find one species of a group deviate so from the central or typical type.

The appearance in the feces of lactobacilli of a type other than those fed was to be expected since they may be normal inhabitants of the intestinal tract, their predominance depending upon the composition of the diet. Porter, Weinstein, and Rettger (1938)

in an investigation to ascertain the bacterial flora of the stomach, segments of the small intestine and cecum of white rats, found lactobacilli present in large numbers throughout. Eppright, Valley and Smith (1937) have shown that salts of calcium and phosphorus, in addition to carbohydrates, were essential for the maintenance of an aciduric flora. Since milk contains all of these we would expect it to favor the development of those aciduric organisms already present as well as those which were being fed, providing the organisms were implantable, and this appeared to occur in our experiments. However, acidophilus milk was much more effective than sterile skimmed milk in increasing the number of those aciduric organisms originally present in the intestinal tract.

Whether the aciduric organisms isolated from rats should be classified as *Lactobacillus acidophilus* or *Lactobacillus bifidus* is another debatable question. Rettger and others have reported isolation and identification of both *L. acidophilus* and *L. bifidus* from rat feces. Other than differences in carbohydrate reactions which are of questionable value for separating members of this genus, the organisms isolated in our study were very much like the smooth (Y) type of *L. acidophilus* and hence we have designated them as such. Possibly some could have been placed in the so called *L. bifidus* groups, except that results already published may not warrant the establishment of two distinct species for organisms so closely related. Weiss and Rettger (1934) (1938) have emphasized the similarity of these organisms and suggest that *L. bifidus* from breast-fed infants and rats be considered a variant of the species *L. acidophilus*.

Another point of interest is the possible significance of aciduric organisms normally present in the intestinal tract. Since these species may be made to predominate by regulation of the diet it would seem that when they are present it might be superfluous to administer other foreign aciduric bacteria. However, in these studies the administration of *L. acidophilus* milk possessed a distinct advantage over sterile milk in stimulating the normal aciduric flora.

Finally, this investigation emphasizes the need for a method of

examining commercial acidophilus preparations beyond mere identification of organisms. Our results indicate that there may exist rough (X) types of *L. acidophilus* which are not implantable in white rats, although they are typical in all other respects. This is in agreement with other investigations which have indicated that some strains of *L. acidophilus* vary in their ability to become acclimated to the intestinal tract. Hence, it seems that the important features of an analytical procedure for acidophilus preparations should be to establish the identity of the organisms and to determine their implantation.

SUMMARY AND CONCLUSIONS

Of the seven oral or dental strains of lactobacilli employed, none were implantable in the intestinal tract of white rats by the procedure used. On the other hand, four of five rough intestinal strains and all of the five smooth intestinal strains were implantable.

Rough intestinal *Lactobacillus acidophilus* organisms (with the exception of one strain) were more readily implanted and persisted for a longer time following the cessation of feeding the organisms than did smooth strains. The one culture of *Lactobacillus bulgaricus* used was not implantable which is in accord with previous studies.

With the exception of the duodenal contents, which contained relatively few organisms, other specimens from sections of the intestinal tract of the animals sacrificed gave results upon microscopic examination and cultivation similar to those obtained with fecal samples.

Fermentation of sucrose, maltose and raffinose by intestinal strains, sucrose and maltose by dental strains and sucrose and mannitol by rat strains occurred with few exceptions. The remaining fermentation reactions were irregular and, thus, fermentation reactions alone could not be used for separation of strains into groups, although in individual instances fermentation results aided in identification of a given strain. The intestinal strains exhibited a slightly higher tolerance to phenol than did

the dental strains, and this was the only biochemical characteristic used which correlated with the results on implantation.

Lactobacilli, apparently rat strains of *Lactobacillus acidophilus*, were isolated from each rat receiving lactobacilli milk cultures as well as from a control rat receiving sterile skimmed milk. This emphasizes the necessity of controlling implantation experiments adequately to insure that the strain isolated is actually the strain administered, especially when smooth strains of lactobacilli are employed.

The results emphasize the errors that are likely to occur in identifying aciduric organisms from the oral cavity as *Lactobacillus acidophilus*. Biochemical differences between intestinal and oral strains have been shown to exist by other investigations, while this work points out the inability of the oral strains studied to become implanted in the intestinal tract of rats. Such evidence may not justify designation of oral strains of aciduric organisms as *Lactobacillus acidophilus* since implantation is a generally accepted characteristic of this species.

The results also indicate the need for improved methods of examining *Lactobacillus acidophilus* preparations to include not only identification of the organisms but in addition whether or not they can be implanted.

REFERENCES

BUNTING, R. W. 1937 The prevention of dental caries. Jour. Mich. State Dental Soc., **19**, 65–69.

BUNTING, R. W., AND PALMERLEE, F. 1925 The rôle of *Bacillus acidophilus* in dental caries. Jour. Amer. Dent. Assoc., **12**, 381–413.

CURRAN, H. R., ROGERS, L. A., AND WHITTIER, E. O. 1933 The distinguishing characteristics of *Lactobacillus acidophilus*. Jour. Bact., **25**, 595–620.

EPPRIGHT, E. S., VALLEY, G., AND SMITH, A. H. 1937 Influence of salts in the diet on the intestinal flora of the albino rat. Jour. Bact., **34**, 81–97.

HADLEY, F. P. 1933 A quantitative method for estimating *Bacillus acidophilus* in saliva. Jour. Dent. Res., **13**, 415–428.

HADLEY, F. P., AND BUNTING, R. W. 1932 Further studies on the recognition of *B. acidophilus*. Jour. Amer. Dent. Assoc., **19**, 28–36.

HADLEY, F. P., BUNTING, R. W., AND DELVES, E. A. 1930 Recognition of *Bacillus acidophilus* associated with dental caries; a preliminary report. Jour. Amer. Dent. Assoc., **17**, 2041–2058.

HOWE, P. R., AND HATCH, R. E. 1917 A study of the organisms of dental caries. Jour. Med. Res., **36**, 481–492.

JAY, P. 1937 Dental caries, a public health problem. Illinois Health Messenger, **9**, 40–44.

JOHNSTON, M. M., WILLIAMS, C. H. M., ANDERSON, P. G., DRAKE, T. G. H., TISDALL, F. F., AND KAAKE, MILDRED J. 1936 *Lactobacillus acidophilus* in dental caries. Jour. Amer. Dent. Assoc., **23**, 1493–1497.

KOPELOFF, LENORE M., AND KOPELOFF, N. 1937 Optical activity of lactic acid produced by *L. acidophilus* and *L. bulgaricus*. Jour. Bact., **33**, 331–334.

KULP, W. L. 1929 Preliminary report on a method for the differentiation of *L. acidophilus* from *L. bulgaricus*. Jour. Bact., **17**, 355–361.

MORISHITA, T. 1929 Studies on dental caries, with special reference to aciduric organisms associated with this process. I. Isolation and description of organisms. Jour. Bact., **18**, 181–198.

PORTER, J. R., WEINSTEIN, L., AND RETTGER, L. F. 1938 The influence of diet on the distribution of bacteria in the stomach, small intestine and cecum. Jour. Bact., **35**, 72.

RETTGER, L. F. 1932 A brief survey of the group of acid-producing and acid-tolerating bacteria known as the *Lactobacillus* genus. Yale Jour. Biol. and Med., **4**, 485–498.

RETTGER, L. F., LEVY, M. N., WEINSTEIN, L., AND WEISS, J. E. 1935. *Lactobacillus acidophilus* and its therapeutic application, pp. 16–25. Yale University Press.

RODRIGUEZ, F. E. 1922 Studies on specific bacteriology of dental caries. Mil. Dent. Jour., **5**, 199–214.

ROSEBURY, T., LINTON, R. W., AND BUCHBINDER, L. 1929 Comparative study of dental aciduric organisms and *Lactobacillus acidophilus*. Jour. Bact., **18**, 395–412.

SABINE, D. B. 1937 Lipolytic activity of the *Lactobacillus acidophilus*. Jour. of Lab. and Clin. Med., **23**, 1183–1184.

ULICNY, H. P. 1936 Some comparative studies of *Lactobacillus acidophilus* associated with dental caries. Jour. Dent. Res., **15**, 397–402.

WEINSTEIN, L., ANDERSON, T. G., AND RETTGER, L. F. 1933 An attempt to dissociate certain oral and dental strains of lactobacilli, and some notes on differentiation of the rough variants from intestinal lactobacilli. Jour. Dent. Res., **13**, 323–328.

WEISS, J. E., AND RETTGER, L. F. 1934 *Lactobacillus bifidus*. Jour. Bact., **28**, 501–522.

WEISS, J. E., AND RETTGER, L. F. 1938 Taxonomic relationships of *Lactobacillus bifidus* (*B. bifidus* Tissier) and *Bacteroides bifidus*. Jour. Inf. Dis., **62**, 115–120.

A STUDY OF THE HEMORRHAGIC SEPTICEMIA PASTEURELLAE

CARLOS T. ROSENBUSCH AND I. A. MERCHANT

Department of Veterinary Hygiene, Division of Veterinary Medicine, Iowa State College, Ames, Iowa

Received for publication July 5, 1938

Early investigators depended for the diagnosis of hemorrhagic septicemia entirely upon the lesions and the bipolar staining and pathogenicity of the organisms encountered as etiologic agents. It is evident, therefore, that in early references, organisms of widely divergent characteristics have been included in the *Pasteurellae* group.

The first report of an organism of this group was by Rivolta in 1877 following his study of fowl cholera. This investigation was followed by several others, establishing a host of organisms as the causative agents of the various hemorrhagic septicemia diseases. Hueppe, in 1886 was the first to observe the close relationship of the various organisms of the group when he used the name *Bacterium septicaemia-hemorrhagica* for the etiologic agents of fowl cholera, cattle, rabbit, and swine septicemia. Kitt, however, in 1885 used the term *Bacterium bipolare-multocidum* to embrace the organisms causing the disease in cattle, swine, deer, horses, sheep, and goats. These two authors were the first exponents of the unicist school. The work of Lignières, however, is probably the basis of the zoologic species classification of the Pasteurellae, even though he recognized the close cultural and biochemic similarities of the various group constituents. Lignières' classification was based almost entirely on the isolation history of the various organisms. This classification has prevailed up to the present, although experimental evidence has pointed to its inadequacy as a method of subdividing the genus.

Baumgarten (1911) was one of the first investigators to doubt the value of the zoologic classification, basing his objection on the

experimental cross-pathogenicity of the various groups or species. Similar conclusions regarding experimental and field cross-pathogenicity, as well as cross-immunity, have been reported by the following investigators: Mohler and Eichhorn (1913), Hutyra (1925), Migge (1933), and Manninger (1934).

Further evidence against the zoologic classification was provided by studies of the cultural, biochemic, and serologic characteristics of the various components of the group. A general tendency to doubt the value of Lignières' classification has resulted, since there is a decided similarity between the various strains and none of the strain differences correspond to the zoologic species subdivision.

On the basis of cultural, biochemic and serologic characteristics, the Pasteurellae have been divided into a typical group and an atypical group by several investigators. Organisms belonging to the latter group isolated from cattle and sheep have been studied by Jones (1921), Spray (1923), Jorgensen (1925), Eddington (1930), Newsom and Cross (1932), and others. Newsom and Cross, in 1932, studied this group in detail and reported it as identical to Jones' Group I, because the two groups are similar in pathogenicity, hemolysis, absence of indol formation, and fermentation of lactose, maltose, inositol, dextrin, and raffinose. These last investigators placed the atypical strains in a separate species which they called *Pasteurella hemolytica*.

The typical group has been studied by investigators in various countries and subdivided into several subgroups or types, which, unfortunately, have not been correlated sufficiently for their classification status to be established. Some of the investigators responsible for the subdivision of this group, which contains most of the hemorrhagic septicemia Pasteurellae are: Koske (1927), Roderick (1922), and Zaisen (1934), all of whom resorted to complement-fixation differences, and Cornelius (1929), Ochi (1934), Yusef (1935), and Khalifa (1936) on the basis of agglutination, agglutinin-absorption, and precipitin-absorption reactions. Khalifa, in 1934, was the first investigator to correlate the serologic results with the fermentation of xylose, arabinose and mannitol.

Culture variations have been observed in the Pasteurellae by many investigators; however, few of the results have been correlated. The earliest report was by Manninger (1919) who described an avirulent, uncapsulated, highly immunogenic culture-variant of avian origin.

Other supposedly rough variants were described by De Kruif (1922) as virulent "D", and avirulent, rough "G" types. Webster and Burn (1926), added to these an "I" or intermediate type and a more stable "M" or mucoid type. Anderson, Coombes, and Mallick, in 1929, initiated the use of "S" and "R" to replace the "D" and "G" types of De Kruif. Brigham and Rettger (1935) used the same terminology and, in addition, recognized the occurrence of "I" or intermediate forms.

Other reports describing cultural variations dealt with fluorescence. Webster and Hughes (1929) described three types on the basis of fluorescence, pathogenicity, and agglutinability. The fluorescent form was highly pathogenic and of poor agglutinability, whereas the non-fluorescent form was of lower pathogenicity. The third type was an intermediate. Morch and Krogh-Lund (1931) and Ochi (1933) reported types that seem to be similar to the three types described above. Cornelius, in 1931, also found a similar change, but he used the terms, "I" form, for the less stable, fluorescent and poorly agglutinable type, and "A" form for the stable non-fluorescent and highly agglutinable type. This author stated that he could obtain "A" forms from his "I" forms, by subjecting the cultures to unfavorable environmental conditions, but he could not do the opposite, for they reverted very rapidly to the original "A" form.

EXPERIMENTAL MATERIALS AND PROCEDURE

One hundred and fourteen strains were studied, but due to the lack of space[1] results for only 44 representative strains are presented in tables 1 and 3. The isolation history of many of the strains, other than the species from which they originated,

[1] For additional complete data refer to C. Rosenbusch's Master's Thesis on the "Biologic and Serologic Relationships of the Hemorrhagic Septicemia Pasteurellae" deposited at the Library of the Iowa State College, Ames. Iowa.

was not available. The origin of the 114 strains studied was as follows: 38 avian, 22 bovine, 18 ovine, 15 porcine, 7 buffalo, 7 equine, 4 rabbit, and one each of deer, cat, and mink. Of the 44 cultures discussed in this paper[1] the source and original numbers are as follows:

1. Strains: 153 (1336-Jones), 159 (Woodbury), 161 (Mountain sheep), 164 (52B), 165 (4277-Jones), 168 (54), 169 (33), 175 (18A), 178 (Hereford), from Dr. I. E. Newsom, Ft. Collins, Colorado.
2. Strains: 104 (8–65), 106 (8–75), 109 (8–58), 116 (8–71), 120 (8–66), 122R (8–54), 130 (8–76), 134 (8–64), 138 (8–70) from the Jensen-Salsbery Laboratories, Kansas City, Missouri.
3. Strains: 70 (170), 84, 95, 217M (117), 257, 335, 642 from the Pittman-Moore Laboratories, Indianapolis, Indiana.
4. Strains: 150, 226, 412, 1525, 4300 from the Fort Dodge Serum Co. Laboratories, Fort Dodge, Iowa.
5. Strains: 232, 234R, 236, 242R, 243 from the Norden Laboratories, Lincoln, Nebraska.
6. Strains: 33R (N33), 590M (2590), 779 (H779) from the United States Bureau of Animal Industry Laboratories, Washington, D. C.
7. Strains: 31, 57, 1932 from the Lederle Laboratories, Pearl River, New York.
8. Strain: 886 from Dr. H. D. Marsh, Bozeman, Montana.
9. Strains: 35M, R, T, 70R from miscellaneous sources.

Morphologic and cultural technic

Preparations stained by dilute carbol-fuchsin and Giemsa stains were examined to detect capsules. A basic medium containing 0.5 per cent proteose-peptone (Difco) and 0.5 per cent sodium chloride, 0.02 per cent di-potassium-phosphate, and 0.01 per cent magnesium sulphate at a pH of 7.2–7.4, or a buffered pepsin-digested beef-infusion medium was used for cultural purposes. A medium containing beef-extract broth, 1.2 per cent agar and 20 per cent serum was used for stock cultures, colony studies, and dye bacteriostasis.

Growth characteristics in broth were observed at daily intervals for the detection of variants. Motility was studied after 24

hours of growth in the same broth cultures. Hemolysis was determined on dilute horse-blood agar plates and recorded after 18 to 36 hours. Bile solubility tests were made by the addition of 0.5 to 1 cc. of heat-sterilized sheep bile to 24-hour cultures.

Dye bacteriostasis tests were carried out in a series of dilutions between 1:10,000 and 1:100,000 of crystal violet, basic fuchsin, thionine, pyronine B, malachite green, and brilliant green. Several cultures were inoculated on the same plate and care was taken to prevent the use of too great bacterial inocula which would otherwise allow growth by superposition of organisms. Readings were taken at daily intervals for five days.

Biochemic and pathogenesis technic

A total of 15 carbohydrates, 6 alcohols, and 1 glucoside were used to study the saccharolytic properties of the organisms. The fermentation tests were made in a medium composed of one-per-cent of the sugar in basic broth with one-per-cent of Andrade's indicator. The media were filter-sterilized through a W Berkefeld candle. Readings were taken daily for 10 days and then at 10-day intervals until the thirtieth day. The fermentation tests were first made using a serum broth which had been heat-sterilized. It gave irregular results, due to heat sterilization or to the action of the serum enzymes, especially on maltose and xylose.

Indol formation was tested on the basic broth medium after 4 or 5 days incubation. A slight modification of Kovacs' test (1928) was used to detect the formation of indol. It consisted of the addition of 1 cc. of ether to each tube, which concentrated the indol at the surface of the medium and allowed a rapid and definite purple indol-ring to form. Nitrate reduction tests were made on 5 day-old cultures in basic broth to which 0.2 per cent sodium nitrate had been added. Hydrogen sulphide and Voges-Proskauer tests were made with Kligler's lead acetate and 2 per cent glucose broth respectively, the latter tested by the addition of 50 per cent caustic potash and read after 24 hours.

Mouse pathogenesis was determined by the intraperitoneal injection of each organism into three mice. The first two in-

jections of 0.5 cc. each were made with 18- to 24-hour broth cultures, while the third test was done with 1 cc. of a dilute suspension of a young serum-agar culture. A series of mouse passages was also carried out with some of the strains to determine the effect of successive animal passages. The dead animals were autopsied and cultures made from the heart to diagnose the cause of death.

Agglutination technic

Twelve rabbits were injected, 25 times each during a period of 90 days, with 12 different broth antigens. The first 15 injections were made intravenously and intraperitoneally with heat-killed cultures, producing homologous titres lower than 1:1,600. In the remaining 10 inoculations live cultures were injected subcutaneously, intraperitoneally, and some intravenously. The final homologous titre of most sera was 1:32,000. There were, however, two strains of very poor antigenic ability which did not cause the production of such a high titre.

The agglutination tests were made with agar-growth antigens suspended in physiologic saline in two series of increasing serum dilutions. The first series consisted of dilutions ranging between 1:25 to 1:3,200 while the second series ranged from 1:250 to 1:32,000. The tests, with their controls, were then placed at a temperature of 40–43°C. and the readings taken after 72 and 96 hours of incubation.

EXPERIMENTAL RESULTS

Morphology and cultural characteristics (see table 1)

The organism proved to be so pleomorphic that it could not be typed on the basis of morphology, nor, at times, distinguished by this means from contaminating forms. Pleomorphicity was most noted in broth and carbohydrate media while uniformity in shape was increased by repeated transfers on solid-medium or animal passage. Carbohydrate media, especially when fermented, were responsible for the greatest pleomorphicity of the organism; there were, in some cases, organisms 3 to 4 times their

normal size as well as long chain formations. Capsule or pseudo-capsule formation was observed on some of the larger cells.

In serum-agar plates fluorescent, non-fluorescent, and intermediate colonies were distinguished. A differentiation in species could not be made on the basis of colony characteristics. The occurrence of fluorescence was not constant but fluctuating and was partially associated with continuous serum-agar transfers or with a series of mouse passages. The changes of colonies toward the fluorescent mucoid type occurred spontaneously in single colonies, but were usually short-lived. In general it was found that the intermediate fluorescent forms were the least stable, while the non-fluorescent forms were the most stable. Recently isolated strains showed the fluorescent characteristic in the majority of cases.

In broth, most of the organisms grew diffusely, but there were some strains that showed an early precipitation into granular or floccose particles, leaving the supernatant fluid clear or almost so. This latter characteristic was later correlated with the avirulence of the culture. A partial sedimentation of a mucoid-stringy nature was noted in a number of the strains. None of the strains were motile.

Two main divisions could be made on the basis of hemolysis. The hemolytic forms corresponded to Newsom and Cross' *Pasteurella hemolytica*, and the remaining strains corresponded to the typical *Pasteurella* group of the same authors.

Bile-solubility tests proved negative for all strains. No satisfactory species or group differentiation could be obtained from the study of dye bacteriostasis with six different dyes. There was, in general, however, a tendency of the avian strains to grow in higher concentrations of crystal violet. The growth-limits of the avian strains ranged between the dilutions of 1:15,000 to 1:25,000 whereas the other strains' growth-limits ranged between 1:33,000 to 1:50,000.

The pH range of the organisms in proteose-peptone broth as tested with brom-thymol blue, chlorphenol red, and phenol red was between 6 and higher than 8.5 with an optimal pH between 7.2 and 7.4.

TABLE 1

Biochemic, cultural and biologic characteristics of 44 representative Pasteurella organisms

STRAINS	HOST ORIGIN	PENTOSES		ALCOHOLS						DI- AND TRISACCHARIDES					HEMOLYSIS	INDOL PROD.	NITRATE RED	BILE SOLUBIL.	MOUSE PATHOGENESIS	COLONY FLUORESCENCE	BROTH CHARACTERISTICS	MORPHOLOGY
		Xylose	Arabinose	Dulcitol	Glycerol	Adonitol	Mannitol	Sorbitol	Inositol	Lactose	Maltose	Sucrose	Trehalose	Raffinose								
Group I																						
104	Avian	−	+	+	±	−		+	−	−	−	+	−	−	−	±	+	−	−	N	D	R
106	Avian	−	+	+	−	−		+	−	−	−	+	−	−	−	+	+	−	+++	F	M	R
335	Avian	−	+	−	+	−		+	−	−	−	+	−	−	−	±	±	−	+++	F	D	I
1525	Avian	−	+	+	+	−		+	+	−	−	+	−	−	−	+	+	−	+++	F	D	R
T	Avian	−	+	+						−	−	+			−	+	+	−	+++	F	D	R
57	Bovine	−	+	+	+	−	+	+	−	−	−	+	−	−	−	+	+	−	+++	F	D	R
226	Bovine	−	+	+	+	−		+	−	−	−	+	−	−	−	+	+	−	++	F	D	I
31	Ovine	−	+	+	+	−	+	+	−	−	−	+	−	−	−	+	+	−	+++	F	D	R
642	Ovine	−	+	+	+	−	+	+	−	−	−	+	−	−	−	+	+	−	++	N	D	I
150	Porcine	−	+	+	−	−	+	+	−	−	−	+	−	−	−	+	+	−	+++	F	D	R
236	Porcine	−	+	+	±	−		+	−	−	−	+	−	−	−	+	+	−	++	N	M	I
257	Equine	−	+	+	−	−		+	−	−	±	+	−	−	−	+	+	−	+	N	D	I
Group II																						
120	Equine	+	−	−	−	−	±	−	−	−	−	+	−	−	−	+	+	−	+++	N	M	I
243	Equine	+	−	−	+	−		+	−	−	−	+	−	−	−	+	+	−	+	N	D	I
84	Bovine	+	−	−	−	−		+	−	−	−	+	−	−	−	+	+	−	++	N	D	R
109	Bovine	+	−	−	−	−		−	−	−	−	+	−	−	−	+	+	−	++	N	M	R
153	Bovine	+	−	−	+	−		+	−	−	−	+	±	−	−	+	+	−	++	F	D	R
1932	Bovine	+	−	−	−	−	+	+	−	−	−	+	−	−	−	+	+	−	+++	F	D	I
116	Buffalo	+	−	−	−	−		−	−	−	−	+	−	−	−	+	+	−	++	N	M	I
779	Buffalo	+	−	−	+	−	+	+	−	−	−	+	−	−	−	+	+	−	+++	N	M	I
4300	Buffalo	+	−	−	−	−	+	+	−	−	−	+	−	−	−	+	+	−	+++	N	M	I
168	Ovine	+	−	−	+	−		+	−	−	−	+	−	−	−	+	+	−	+++	F	D	R
169	Ovine	+	−	−	+	−		+	−	−	−	+	−	−	−	+	+	−	+++	F	M	R
175	Ovine	+	−	−	−	−		+	−	−	−	+	−	−	−	+	+	−	++	F	D	R
134	Porcine	+	−	−	−	−		+	−	−	−	+	−	−	−	+	+	−	+++	F	M	I
70	Porcine	+	−	−	−	−		+	−	−	−	+	−	−	−	+	+	−	+++	N	D	I
70R	Porcine	+	−	−	−	−		+	−	−	−	+	−	−	−	+	+	−	−	N	M	R
138	Rabbit	+	−	−	−	−		−	−	−	−	+	−	−	−	+	+	−	+++	N	D	R

Mouse Pathogenesis: (+++) killing in 6–9 hours; (++) in 10–15 hours; (+) in 16 or more hours.

Fluorescence: (F) fluorescent; (N) not fluorescent.

Carbohydrates: (+) fermented; (±) slight fermentation; (−) not fermented.

Broth characteristics: (D) diffuse; (M) mucoid precipitate; (P) complete precipitation.

Morphology: (R) regular; (I) irregular.

(•) Doubtful classification.

TABLE 1—*Concluded*

STRAINS	HOST ORIGIN	PENTOSES		ALCOHOLS						DI- AND TRISACCHARIDES						HEMOLYSIS	INDOL PROD.	NITRATE RED	BILE SOLUBIL.	MOUSE PATHOGENESIS	COLONY FLUORESCENCE	BROTH CHARACTERISTICS	MORPHOLOGY
		Xylose	Arabinose	Dulcitol	Glycerol	Adonitol	Mannitol	Sorbitol	Inositol	Lactose	Maltose	Sucrose	Trehalose	Raffinose									
Group III																							
95*	Ovine	−	+	+	±	−		+	−	−	±	+	−	−	−	+	+	−	−	N	D	R
130	Ovine	+	+	+	−	−		+	−	−	−	+	−	−	−	+	+	−	++	F	M	R
412	Ovine	+	+	+	+	−	+	+	−	−	−	+	−	−	−	+	+	−	++	F	D	R
R	Bovine																		++	F	D	R
Group IV—Pasteurella hemolytica																							
161	Ovine	−	−	−	−	−			−	+	+	+	+	+	+	−	−	−	−	N	M	R
164	Ovine	+	−	−	+	−		+	+	+	+	+	−	−		−	+	−	−	F	M	I
884	Ovine	±	±	±	+			+		+	±	+	+					−	+	F	D	I
159	Bovine	+	±	±	+	−		+	+	±	+	+	−	+	+	−	+	−	−	N	P	I
165	Bovine	+	±	±	+	−		+	+	+	+	+	−	+	+	−	+	−	−	N	P	R
178	Bovine	+	−	−	+	−		+	+	+	+	+	+			−	±	−	++	F	D	I
Variants																							
35M	Porcine	+	−	−						−	−	+	−		−	+		−	+++	F	D	R
217M	Bovine	−	−	−	−	−		±	−	−	−	+	−	−		+	+	−	−	F	M	I
590M	Bovine	+	−	−	+	−		+	−	−	−	+	+	−	−	+	+	−	+	F	P	R
33R	Ovine	+	−	−	+	−		+	−	−	−	+	−	−	−	+	+	−	−	N	P	R
234R	Ovine	±	−	±	±	−		+	−	−	−	+	±	−	−	+	+	−	−	N	P	I
242R	Buffalo	+	−	−	−	−		±	−	−	−	+	−	−	−	+	+	−	−	N	P	R
122R	Feline	+	−	−	−	−		±	−	−	−	+	−	−	−	+	+	−	−	N	P	I

Biochemic and pathogenesis characteristics (*see table 1*)

The fermentation study of 22 carbohydrates, alcohols and glucosides revealed that xylose, arabinose, and dulcitol were sufficient for the classification of the typical non-hemolytic strains into two main groups. The atypical hemolytic forms could be separated from the rest by their ability to ferment inositol, lactose, maltose, trehalose, and raffinose. This group, which corresponded to Jones' type I and Newsom and Cross' *Pasteurella hemolytica* was classified as group IV. The other carbohydrates and alcohols were carried as contamination controls. The three main carbohydrates were tested a total of 3 to 8 times per culture depending on the strain in use. The results obtained warrant

the sub-division of the typical forms into two main groups: Group I ferments arabinose and dulcitol but not xylose, which corresponds to Khalifa's type A; group II ferments xylose but not arabinose nor dulcitol, which corresponds to Khalifa's type C. Group III included three strains which fermented all three carbohydrates and an additional strain, 95, of doubtful classification, but which showed the fermentation characteristics of group I.

In addition to the carbohydrates and alcohols reported in table 1, the hexose carbohydrates glucose, levulose, galactose, and mannose were fermented by all the strains. The pentose rhamnose, the glucoside salicin, and the polysaccharides inulin, starch, and dextrin were not fermented by any of the typical strains.

The fermentation of xylose occurred somewhat slowly, while that of arabinose occurred more rapidly. A uniformity in the fermentation of arabinose and dulcitol was observed in almost all of the strains. Dulcitol showed somewhat variable results. It apparently inhibited the growth of the organisms, due, possibly, to heat sterilization and prolonged incubation before inoculation.

Four strains, group I cultures 232 and 236, and group II cultures 84 and 1932, which were studied through a period of two years, showed changes in the fermentation of xylose, arabinose, and dulcitol. These organisms were tested a total of 4 to 8 times and the results were quite surprising (see table 2), as they alternated through the experiment from the characteristics of groups I to II.

The variations in fermentation of arabinose and xylose were adequately controlled by comparisons with the other normally-acting cultures and by the constant check on such contaminant-control carbohydrates as lactose, maltose, and sucrose, as well as by additional control tests with raffinose, trehalose, rhamnose, inositol, indol production, colony characteristics, mouse pathogenesis, and dye bacteriostasis. Further evidence that the biochemic variability was not caused by contamination was provided by the constant uniformity in the fermentation of dulcitol and arabinose, which, at the same time, provided evidence that the chemical nature of the carbohydrates had not changed.

Mouse pathogenesis was of little value in the separation of the strains into groups or species. The strains belonging to group IV and those of the variant group were either entirely apathogenic or of low virulence.

In general, no relation could be found between colony characteristics and virulence. On the other hand, when four serial mouse passages were carried out on a bovine strain, 1932, and an ovine strain, 31, there was an increase in colony fluorescence and in virulence to the extent that they caused the death of two chickens.

TABLE 2

Biochemic fermentation variations

STRAINS		TESTS										ORIGINAL CARBOHYDRATE GROUPS	FINAL CARBOHYDRATE GROUPS
		1	2	3	4	5	6	7	8	9	10		
Bovine 232	Arabinose		0	0					+	+		II	I
	Xylose		+	+					0	0			
Porcine 236	Arabinose		0	0					+	+	+	II	I
	Xylose		+	+					0	0	0		
Bovine 84	Arabinose	0	0	+	+				+	±	0	II	II
	Xylose		+	0	0				0	+	+		
Bovine 1932	Arabinose		0	0		+	+	+	+	±	0	II	II
	Xylose		+	+		0	0	0	+	+	+		
Dates of tests.....		2/35	5/35	7/35	9/35	10/35	11/35	12/35	7/36	8/36	1/37	1/37	1/37

(+) = fermentation in less than 8 days; (±) = fermentation in more than 8 days; (0) = no fermentation.

There were marked stable variations in virulence in some individual strains. Strain 642 was completely avirulent and strain 226 was apathogenic in a number of trials, but both strains gradually became more and more virulent. The reverse was true with strains 217 and 259.

Serologic characteristics

Some of the strains revealed an auto-agglutinating tendency. This occurred for the most part in strains which showed poor growth on common agar and in strains showing early broth pre-

TABLE 3

Classification of 44 representative Pasteurellae on the basis of selective agglutinations

STRAINS	HOST ORIGIN	GROUP I					GROUP II			GROUP III		VARIANTS		RABBIT NORMAL	BOVINE SERUM
		Av. 106	Bv. 57	Bv. 226	Su. 150	Eq. 259	Su. 70	Ov. 168	Bf. 779	Ov. 95	Ov. 412	Ov. 234	Su. 35M		
Group I															
104	Avian	+++	+++	+++	+++	+++	+	+++	++	+++	++	+	+	+	+
106	Avian	+++	+++	+++	+++	+++	+	+++	++	+++	++	+	+	+	+
335	Avian	++	+++	+++	+++	+++	+	+++	++	+++	+	−	+	−	+
1525	Avian	++	+++	+++	+++	+++	+	+++	++	+++	+	+	+	+	+
T	Avian	++	+++	+++	+++	+++	+	+++	++	+++	+	+	+	+	+
57	Bovine	++	+++	+++	+++	+++	+	+++	+++	+++	+	+	−	−	+
226	Bovine	++	+++	+++	+++	+++	+	+++	+++	+++	+	+	+	+	+
31	Ovine	++	+++	+++	+++	+++	−	++	++	+++	+	−	−	−	+
642	Ovine	++	+++	+++	+++	+++	−	+++	+	+++	+	−	−	−	+
150	Porcine	++	+++	+++	+++	+++	+	++	+	+++	+	+	+	+	+
236	Porcine	++	+++	+++	+++	+++	+	++	+	+++	+	+	+	+	+
257	Equine	+	+++	+++	+++	+++	+	++	++	+++	+	+	+	+	++
Group II															
120	Equine	++	+	+	+	+	+++	+++	++	+++	++	+	+	+	++
243	Equine	−	+	+	+	+	+++	+++	+++	+	+	+	+	+	++
84	Bovine	+	+	+	+	+++	+++	+++	+++	+	+	+	−	−	++
109	Bovine	+	+	++	+	+++	+++	+++	+++	+	++	+	−	−	++
153	Bovine	+	+	+	+	+++	++	+++	+++	−	+	+	+	−	++
1932	Bovine	+	+	++	+	++	+++	++	+++	+	++	+	+	+	+
116	Buffalo	++	+	++	+	+	+++	+++	+++	+	++	+	+	+	++
779	Buffalo	+	+	+	+	+	+++	+++	+++	+	+	+	+	+	++
4300	Buffalo	+	+	+	+	+	+++	+++	++	+	+	+	+	+	++
168	Ovine	+	+	+	+	++	+++	+++	+++	+	+	−	+	+	++
169	Ovine	+	+	+	−	++	+++	+++	+++	+	+	+	+	−	+
175	Ovine	+	+	++	++	+	+++	+++	+++	++	+	−	−	−	+
134	Porcine	+	++	+	+	+	+++	+++	+++	+	++	+	+	+	++
70	Porcine	+	+	+	+	+	+++	+++	+++	+	+	+	+	+	+
70R	Porcine	+	+	+	+	+	+++	+++	+++	+	+	+	+	+	++
138	Rabbit	+	+	++	++	+	++	++	+	+	+++	+	+	+	+

70R	Porcine	+	+	+	+	+	+++	+++	+++	+	+	+	+	+	++
138	Rabbit	+	+	++	++	+	++	++	+	+	+++	+	+	+	+

Group III

95*	Ovine	+	+	+	+	+	+	−	+	+++	−	−	−	−	+
130	Ovine	+	+	+	+	+	+	+	−	+	+++	+	−	−	+
412	Ovine	+	+	−	+	+	−	+	+	+	+++	+	+	−	+
R	Bovine	+	+	++	+	+	−	++	−	++	+++				

Group IV—Pasteurella hemolytica

161	Ovine	−	−	+	+	−	+	+	−	+	+	−	−	−	+
164	Ovine	+	+	+	+	+	+	+	+	+	+	+	+	+	++
884	Ovine	−	+	−	−	+	+	+	+	−	+	−	+	−	+
159	Bovine	+	+	+	+	−	+	+	+	+	+	+	+	+	+
165	Bovine	+	+	+	+	+	+	+	−	+	+	+	+	+	++
178	Bovine	+	+	+	−	+	+	+	−	+	+	+	+	+	+

Variants

35M	Porcine	+	+	−	−	+	+	+	+	+	−	+	++	−	+
217M	Bovine	+	+	+	−	+	+	++	+	+	+	+	+	+	+
590M	Bovine	+	+	−	−	+	+	+	−	+	+	+	+	+	+
33R	Ovine	−	+	+	−	+	+	+	−	+	−	+	−	−	−
234R	Ovine	+	+	−	+	+	+	+	+	+	++	+	+	−	+
122R	Feline	+	+	+	+	++	+	+	+	+	+	+	+	+	+

(+) = 1:50-1,600; (++) = 1:2,000-8,000; (+++) = 1:16,000-32,000.

(Av.) = aviseptica serum; (Bv.) = boviseptica serum; (Su.) = suiseptica serum; (Ov.) = oviseptica serum; (Eq.) = equiseptica serum; (Bf.) = bubaliseptica serum. (*) = doubtful classification.

cipitation. To decrease this effect, the antigens of these strains were prepared with a lower salt concentration, and the tests were read at more frequent intervals.

By using ten immune sera it was possible to classify 44 *Pasteurella* strains into 4 groups (see table 3) by means of the agglutination test. For that purpose 5 group I sera, 3 group II sera, and 2 group III sera were used. The groups observed corresponded to the carbohydrate grouping mentioned previously.

Group I strains gave the highest agglutination titres with 5 group I sera. Some of the strains were agglutinated by 2 group II sera and especially by one which was considered of intermediate character. These strains were also agglutinated by 1 group III serum, 95, which was the least typical of the group.

Group II organisms were more specific in their agglutination reactions, since they were agglutinated in the higher titres only by the group II sera. Individual agglutination variations were encountered in this group more often than with group I strains. Certain of the equine strains showed a tendency to agglutinate at high titres with group III serum 95. Some of the bovine and ovine strains tended to agglutinate at higher titres with group I serum 259, while porcine strain 134 agglutinated to a moderate titre with group I strain 57, which was otherwise specific to group I. The rabbit strains appeared to vary most from the typical form; they acted as intermediates to group III.

Group III includes 3 organisms of miscellaneous characteristics which gave a high agglutination titre with serum 412. Strain 95 was homologous in agglutination characteristics and acted otherwise as an intermediate variant of group I.

Group IV was composed of serologic strains bearing no relation to any of the sera. The organisms of this group corresponded to Newsom and Cross' *Pasteurella hemolytica* species, for which, unfortunately, no homologous serum was available.

The variant group which was set apart because of its cultural characteristics was shown to contain organisms of low agglutinability and poor antigenicity. Sera 35M and 234R reached the final homologous agglutination titre of 1:2,000 and 1:800 respectively, even though culture 35M was very pathogenic.

Two control sera were used. One was from a normal rabbit of the same origin as most of the rabbits used during the experiment. Another was from a rabbit immunized with *Brucella abortus*. Both these sera showed agglutination titres of certain strains ranging from 0 to 1:800. The animals showed no history of a previous infection so that all the agglutinations under 1:1,600 were considered as insignificant in the interpretation of agglutination results. Since the results of both sera were comparable, only one is included in table 3.

The other serum was a composite *Brucella-abortus*-positive cow-serum. This serum showed fairly high titres with the group II organisms, as well as with two group IV and one group I equine strain.

Cross-agglutination tests on the rabbit sera with a polyvalent *Brucella abortus* antigen were negative. Similarly, tests were made with all the sera on 6 *Salmonella* and 2 *Escherichia* strains with negative results, even though some of the agglutination titres were as high as 1:200, which was attributed to an acquired immunity of the rabbits tested.

Variability

During the course of the study of 114 *Pasteurella* cultures, eight strains were found which were considered to be variants on the basis of a granular or mucoid precipitate in broth cultures, lack of virulence for mice and poor agglutinability with immune sera.

Of these organisms, strain 33R, was a permanent "R" form. Its "S" pathogenic form was available as culture 169, which was provided by Newsom. Both of these strains differed in every respect except their carbohydrate fermentation behavior.

Strain 35M,[2] on the other hand, behaved as a pathogenic

[2] Culture 35M appeared similar to the virulent capsulated forms described by Priestley (Brit. Jour. Exp. Path., 17: 374–378, 1936). The behaviour of this culture as well as some of the other recently isolated organisms of avian origin seemed to conform to this type. According to the above author, the poor antigenicity and agglutinability of such strains are due to the presence of a capsule which is present when the organism is virulent and recently isolated.

and poorly antigenic mucoid "M" form originating from a recent Argentine field case isolation.

The other variants included strains 234R, 112R, and 242R, as well as strains 217M and 590M. These cultures presented varying degrees of irregularity in carbohydrate fermentation and agglutination behavior, which were attributed to their different degrees in phase changes. These conclusions were based upon colony and broth characteristics, and mouse pathogenesis. These organisms represented possible "R", "M" and intermediate forms.

An additional variant studied included strain 70R which was isolated from "S" to form 70. This culture differed from its original culture in rough colony characteristics, precipitation in broth, and lack of mouse pathogenesis. Yet, it was similar serologically and biochemically, and it reverted once after the second month of isolation so that it probably represented an intermediate of the type "SR".

DISCUSSION

The purpose of this investigation was to correlate all the available literature on the inter-relationship of the hemorrhagic septicemia Pasteurellae and to test the validity of the zoologic classification and improve upon the various typing techniques which have been reported.

The biochemic, cultural, biologic, and serologic results revealed that the Pasteurellae were composed of widely differing strains of organisms which warranted their classification into two separate species, as suggested by Newsom and Cross. These authors, in 1932, gave the species name *Pasteurella hemolytica* to an atypical, avirulent, hemolytic, and non-indol-forming group composed of strains of bovine and ovine origin. The second group, which they termed the typical group, was of great homogeneity and included strains from avian, bovine, ovine, porcine, equine, buffalo, rabbit, cat, mink, and deer origin. The origin of these strains, however, could not be detected experimentally by either morphologic, cultural, biochemic, or serologic methods.

The main argument for the host-specificity classification of the group up to now has been epidemiologic. Still, this argument has never been adequately nor sufficiently sustained either epidemiologically or experimentally. On the other hand, many epidemiologic reports, cross-pathogenesis studies and cross-immunity tests support the inter-relation of the various species. Of importance are the reports of Baumgarten and Migge, who have gone so far as to state, that epidemiologically the Pasteurellae, with their various changes and types, follow a definite cyclic development in their adaptation to the various hosts. Similar conclusions are encountered throughout the literature. Most of these reports emphasize that cross-pathogenicity is a factor related to differential virulence and host adaptation.

On the basis of the bacteriologic results obtained, on the well-grounded historical epidemiologic data of the species unity, as well as the similar pathology of the disease in various animals, it is possible to group all the typical Pasteurellae in a single species possessing the characteristics given by Lignières (1900), Vorloud (1908), and Manninger (1934). In conformity with these facts, the name *Pasteurella multocida*, Kitt, 1885, *n. comb.* is suggested to include all of the typical, indol-producing, non-hemolytic hemorrhagic septicemia organisms. The name originates from the first one given to an organism involving more than one host. It is also one of the first names given to the microörganism, and though originally a bi-nomial, it is the most suitable. This grouping is not the first one, since Hueppe, in 1886 (cited by Tonaka, 1926), and Hutyra (1928), used single species names, also bi-nomial in character, to identify the organism.

The typical forms were consistently subdivided by their agglutination and carbohydrate fermentation tests into two main groups, and possibly a smaller, third group, all of which definitely crossed the host species lines. These groups differed in the fermentation of xylose, arabinose, and dulcitol and in agglutination reactions.

Group I contained all of the avian strains as well as representative strains of various other hosts. This group was highly

homogeneous, containing a predominance of the organisms with a fluorescent type of colony, a stable, diffuse appearance in broth, and a tendency toward a regular cell morphology.

Group II contained strains of all origins except avian. It could be subdivided serologically into three or four subgroups depending on the degree of relationship to the members of group I. Strain 70 was possibly the true exponent of the group, while strain 168 was quite closely related to group I. The intermediate character of many of the strains of this group was revealed by the predominance of non-fluorescence, mucoid appearance in broth, and tendency toward an irregular cell morphology.

Group III strains did not show a uniform fermentation of the basic carbohydrates. Three of the strains fermented xylose, arabinose, and dulcitol. The organisms showed only a high-titre homologous agglutination and they were devoid of mouse pathogenicity; they possibly represented intermediate forms between groups I and II. Most of the strains possessed fluorescent colony characteristics, a diffuse broth growth, and a regular cell morphology. It may be of speculative interest to note that the apparent grouping is based only upon the characteristics which the various strains revealed at the time they were studied, and are representative of cyclic changes which the organism passes through under different environments. These changes may be due to the acquisition or loss of certain antigenic fractions of the organism.

It was not possible to differentiate host species nor groups on the basis of dye bacteriostasis. Avian strains of both American and Argentine sources, which appeared to be the most homogeneous and stable, were the most resistant to crystal violet.

Bacterial variations, both of a temporary and a permanent nature, were observed. The former occurred as spontaneous, short-lived changes in fluorescence, often environmental in nature, or in mouse pathogenesis or carbohydrate fermentation, suggesting cyclic changes. The bacterial variations of permanent nature were classed as rough "R" and mucoid "M" forms. Intermediate forms were also encountered. All of these

types of variations have been described in the literature, but their interpretation was difficult, because adequate inter-relating studies were absent. It is believed, therefore, that a careful study of the cultural, morphologic, biochemic, and serologic variations would be of great value in interpreting the changes which the organism exhibits *in vitro* and *in vivo* through long periods of time.

CONCLUSIONS

1. Two distinct types of organisms were studied; they differed in cultural, biochemic, serologic and pathogenic characteristics. One type included typical strains usually associated with hemorrhagic septicemia; the other included atypical forms designated as *Pasteurella hemolytica* by Newsom and Cross.

2. The typical strains were divided into two rather distinct subgroups, and a third, less distinct one, on the basis of xylose, arabinose and dulcitol fermentation and by agglutination reactions.

3. Evidence presented invalidates the present zoologic species classification. The name *Pasteurella multocida* Kitt, 1885, *n. comb.*, which includes all typical strains, is suggested to take the place of all the host species names, which are now in common use.

4. Two types of variability were encountered. The permanent variants were of "R" and virulent "M" types, with their intermediate forms. The temporary variants which were produced in many respects by environmental effects on cultural characteristics, mouse pathogenesis, and biochemic reactions, suggested possible cyclic changes in the organism; these changes may account for the variability of results obtained by many previous investigators.

REFERENCES

ANDERSON, L., COOMBES, M. AND MALLICK, S. 1929 Dissociation of the *Bacillus avisepticus*. Part I and II. Indian Jour. of Med. Res., **17**: 611–639.

BAUMGARTEN, P. 1911 Bacillengruppe der Septicaemia haemorrhagica. Lehrbuch der pathogenen Bakterien. P. 349. Hirzel, Leipsig.

BRIGHAM, C. D. AND RETTGER, L. F. 1935 A systematic study of the Pasteurella genus and certain closely related organisms. Jour. Inf. Dis., **56**: 225–237.

CORNELIUS, J. T. 1929 An investigation of the serological relationships of 26 strains of pasteurella. Jour. of Path. & Bact., **32**: 355–364.

CORNELIUS, J. T. 1931 Some observations on bacterial variation in a strain of *P. suiseptica*. Indian Jour. of Med. Res., **18**: 1167–1176.

DE KRUIF, P. H. 1922 Mutation of the bacillus of rabbit septicemia. Jour. Exp. Med., **35**: 561–573 and 621–633.

EDDINGTON, J. 1930 Pneumonia of bovines due to *Pasteurella boviseptica*. J. Com. Path. & Therap., **43**: 234–252.

HUTYRA, F. 1928 Septicaemia haemorrhagica. Kolle, W. and Wassermann, A. Handbuch der pathogenen Mikroörganismen. Vol. 6, part **1**: 483–528.

JONES, F. 1921 A study of *Bacillus bovisepticus*. Jour. Exp. Med., **34**: 561–577; Jour. Am. Vet. Med. Assoc., 60; n.s., **13**: 271–280.

JORGENSEN, G. E. 1925 Some studies of *Pasteurella boviseptica*. Cornell Veterinarian **15**: 295–302.

KHALIFA, I. A. B. 1934 Pasteurellae in animals and their interclassification. Bul. Minist. Agric. Egypt. 147, 36 p. Abstract in the Vet. Bul., **6**: 792. 1936.

KOSKE 1904–1905 Zur Frage der Ubertragbarteit der Schweineseuche auf Geflügel und der Geflügelcholera auf Schweine durch Verfutterung. Arb. a. Kais. Gesundheitsamt., **22**: 503–526. (Original not seen.) Citation by Lal, R. B., Amer. Jour. Hyg., **7**: 573. 1927.

KOVACS, N. 1928 Eine vereinfachte Methode zum Nachweis der Indolbildung durch Bakterien. Zeits. f. Immunitätsf. und Exp. Ther., **55**: 311–315.

LIGNIÈRES, J. M. 1900 Contribution à l'étude et à la classification des septicémies hémorrhagiques. Les Pasteurelloses. Ann. Inst. Pasteur, **15**: 734–736.

MANNINGER, R. 1919 Ueber eine Mutation des Geflügelcholera-bazillus. Centralbl. Bakt. I. Abt. Orig., **83**: 520–528.

MANNINGER, R. 1934 Considérations critiques sur l'étiologie et la prophylaxie de la septicémie hémorrhagique. Office Internat. des Epizootics, R. 45 (Budapest) 38 p.

MIGGE 1933 Das Virus der Wild und Rinderseuche. Berl. Tierärtzl. Wchschr. **49**: 821–824.

MOHLER, J. R. AND EICHHORN, A. 1913 Immunization against hemorrhagic septicemia. Amer. Vet. Rev., **13**: 409–418.

MORCH, J. R. AND KROGH-LUND, G. 1931 Untersuchungen über die Bakterien der Pasteurellagruppe. Zeits. Hyg. u. Infektskr., **112**: 471–491.

NEWSOM, I. E. AND CROSS, F. 1932 Some bipolar organisms found in pneumonia in sheep. Jour. Am. Vet. Assoc. 80: n.s., **33**: 711–719.

OCHI, Y. 1931 and 1933 Studies on hemorrhagic septicemia organisms, especially on their variability. 4 Reports. Jour. Jap. Soc. Vet. Sci., **10**: 331–349, 350–362, 363–366; and **12**: 47–52.

OCHI, Y. 1934 Studies on hemorrhagic septicemia organisms. Report V and VI. Correlations between hemorrhagic septicemia organisms isolated from various animals. Jour. Jap. Soc. Vet. Sci., **12**: 185–199, 1933; and **13**: 163–180.

RODERICK, L. M. 1922 Some antigenic relations of the *Bipolaris septicus* group of bacteria. Jour. Inf. Dis., **31**: 313–325.

SPRAY, R. S. 1923 Bacteriologic study of pneumonia in sheep. Jour. Inf. Dis., **33**: 97.

TANAKA, A. 1926 A comparative study of Pasteurella cultures from different animals. Jour. Inf. Dis., **38**: 421–428.

VOURLOUD 1908 Action de quelques bactéries sur les hydrates de carbon, et le lait tournesolé. Centralbl. Bakt. I. Abt. Orig., **45**: 193–205.

WEBSTER, L. T. AND BURN, D. G. 1926 Biology of Bacterium lepisepticum. Physical, cultural, growth characteristics and virulence of diffuse and mucoid types and their variants. Reports III and IV. Jour. Exp. Med., **44**: 343–358 and 359–386.

WEBSTER, L. T. AND HUGHES, T. P. 1929 The epidemiology of fowl cholera. Reports I and II. Jour. Exp. Med., **51**: 219–223 and 225–238.

YUSEF, H. S. 1935 Serological classification of Pasteurella strains. Jour. Path. and Bact., **41**: 203–206.

ZAISEN, K. 1934 Serological studies on hemorrhagic septicemia organisms, especially on their complement-fixation reaction. Jour. Jap. Soc. Vet. Sci. (English abstract), **13**: 181–191.

CHROMOGENIC STRAINS OF ESCHERICHIA[1]

RALPH P. TITTSLER

Department of Bacteriology, University of Rochester, School of Medicine and Dentistry, Rochester, New York

Received for publication July 5, 1938

Chromogenic strains of the genus *Escherichia*, obtained from human feces and from water, have been described briefly in a preliminary report (1937). Except for the production of a "reddish-orange" pigment, these strains cannot be distinguished from certain well-established non-chromogenic species of *Escherichia*. On the basis of minor differences, they fall into three types. They form gas, produce indol, yield negative Voges-Proskauer reactions, and fail to liquefy gelatin. Thus, they are distinctly different from most of the "yellow" pigment-forming "colon" or "colon-like" organisms which have been isolated from cereals, water, *etc.* by other workers, and which have been mentioned by Parr (1937), and by Lehmann, Neumann and Breed (1931). On the other hand, the present strains may be identical with some of those encountered previously. This point cannot be settled, because the descriptions which have been recorded in the literature are incomplete, except for those of Parr (1937). Two of the present strains seem to be identical with the culture described by him.

The present paper records in detail the characteristics of five strains of chromogenic coliform bacilli and compares them with well-recognized species of *Escherichia*.

MATERIALS AND METHODS

Three of the bacterial strains were isolated in 1932 from eosin methylene-blue agar during the course of routine bacteriological

[1] This work was supported in part by contributions from the General Education Board Research Fund and the Department of Biology, College of Arts and Science, University of Rochester.

examinations of feces and water, and were considered to be typical members of the genus *Escherichia* until they were cultivated on nutrient agar. One strain was obtained in 1936 from the feces of an adult who had suffered for years from chronic constipation. On eosin methylene-blue agar and on Endo's medium, inoculated directly from a fecal suspension, it also appeared to be a typical member of the *Escherichia*, but on blood agar it was distinctly chromogenic. Several isolations were made from each medium and each subculture proved to be chromogenic on nutrient agar. One strain was made available through the courtesy of Dr. R. S. Breed, who had received it from Dr. A. J. Kluyver of Delft, Holland. Originally, it came from Dr. M. W. Beijerinck's collection and "had been carried in the Delft collection for a long time, possibly for as long as 20 years." The original source is not known.

The media and methods employed to determine the various characteristics were the same as those described by Tittsler and Sandholzer (1935). Hemolysis was determined in pork-infusion agar containing rabbits' blood, according to the method of Paulson and Brown (1931).

From 3 to 6 subcultures were isolated from each parent strain, and the cultural characteristics were determined for each subculture. During the course of the present study all of the characteristics were redetermined at least once.

RESULTS

The results of a detailed study of the cultural characteristics of five strains of chromogenic coliform bacilli are recorded, for the most part in tables 1 and 2. All of the strains were found to possess certain features in common. These are shown in table 1. They fermented glucose and lactose with the production of acid and gas; failed to liquefy gelatin; produced indol; gave positive methyl-red tests; and yielded negative Voges-Proskauer reactions. Carbohydrates were fermented rapidly, with the exception of salicin and glycerol, in which acid formation did not occur until the second or third day of incubation.

These cultures differed among themselves in certain char-

acteristics, as shown in table 2. On the basis of these differences, the five strains fell into three types. Strains 219 and 870, from water and from human feces respectively, were alike. Strains 220 and 251 were alike and, evidently, they were identical with the culture described by Parr (1937). Strain 221 differed from the others.

TABLE 1

Characteristics common to all five strains of chromogenic coliform bacilli

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS	CARBOHYDRATES FERMENTED WITH ACID AND GAS	CARBOHYDRATES NOT FERMENTED
Bacilli	l-Arabinose	Sucrose
Non-sporogenous	d-Galactose	Cellobiose
Gram-negative	d-Glucose	Raffinose
Aerobic	d-Levulose	*a*-Methylglucoside
Gelatin not liquefied	d-Mannose	Dulcitol
Indol produced	d-Xylose	l-Erythritol
Nitrates reduced	d-Lactose	l-Inositol
Methyl-red positive	Maltose	Inulin
Voges-Proskauer negative	Trehalose	Starch
H_2S not produced	Glycerol	
Citrate not utilized	Mannitol	
Milk acidified, coagulated and reduced	Sorbitol	

TABLE 2

Differential characteristics of five strains of chromogenic coliform bacilli

	221	220	251	219	870
Salicin	AG	AG	AG	–	–
Adonitol	AG	–	–	AG	AG
Hemolytic type	Beta	Gamma	Gamma	Beta	Beta
Motility	+	+	+	–	–
Source	Feces	Feces	Unknown	Water	Feces

AG = Acid and gas.

The color of the pigment produced by the parent cultures was designated by the writer as "reddish-orange" (1937). When classified according to Ridgway's Color Standards (1912), however, it is xanthine orange. Parr characterized the pigment produced by his strain as "a distinct golden-brown" and "practically the same color as that observed for a typical *Staphylococcus*

aureus." When strains 219 and 221 were plated on nutrient agar, they usually yielded a few colonies which had very little color, but subcultures from such colonies formed as much pigment as did the parent strains. On the other hand, strains 220 and 870 yielded some colonies which were practically non-chromogenic and subcultures from them developed but little pigment. When these slightly chromogenic substrains were plated and isolations made from the least chromogenic colonies, cultures were obtained which develop no detectable pigment in colonies and very little in mass growths. Otherwise, the slightly chromogenic strains did not differ from the most chromogenic substrains of the same parent culture. All of the parent strains have retained their chromogenicity constantly during repeated cultivation on nutrient agar, for as long as six years.

DISCUSSION

The results of a detailed study of the cultural characteristics of five chromogenic strains of coliform bacilli show that they are more closely related to members of the genus *Escherichia* than to those of any other genus. On the basis of chromogenesis alone, they might be allocated to either of the genera, *Flavobacterium* or *Serratia*. This designation would seem to be illogical, however, because their failure to liquefy gelatin, their fermentation of numerous carbohydrates with the formation of gas, and their production of indol, are different from those recorded for members of either of these genera. When the characteristics of the present strains are compared in detail with those recorded in Bergey's Manual (1934) for certain members of the genus *Escherichia*, one finds a close agreement, except for the production of pigment. Thus, strains 219 and 870 differ from *Escherichia acidilactici*, and strains 220 and 251 from *Escherichia paragruenthali*, only in their inability to ferment raffinose. Strain 221 differs from *Escherichia paragruenthali* in its ability to ferment adonitol and in its inability to ferment raffinose. The writer, however, has found that raffinose is not fermented by members of the *Escherichia* genus which do not ferment sucrose, *i.e.*, not by members of the coli-communis group. Thus,

except for chromogenesis, strains 220, 221 and 251 would be classified as *Escherichia paragruenthali* and strains 219 and 870 as *Escherichia acidilactici*. Strains 220 and 251 are identical with the strain described by Parr (1937) and for which he proposed the name *Bacterium aurescens, n.s.*, because it differed from *Escherichia paragruenthali* "not only in being chromogenic but in failing to ferment raffinose." Whether this proposal be acceptable or not depends upon the point of view. If strains like 220 and 251 are given a specific name, the argument can be raised that strains 219 and 870, and 221 deserve similar consideration on the basis of differences in the fermentation of salicin and adonitol and in hemolysis. The writer does not favor the segregation of closely related strains into new species, unless the procedure serves a useful purpose or cannot be avoided. Accordingly, no additional specific names are proposed for the present chromogenic strains. It seems sufficient to consider them as chromogenic varieties of either *Escherichia paragruenthali* or *Escherichia acidilactici*, depending upon their fermentation of salicin.

SUMMARY

A detailed study of the cultural characteristics of five strains of chromogenic coliform bacilli, isolated from water and human feces, is presented. On the basis of differences in the fermentation of salicin and adonitol and in hemolysis, these strains fall into three distinct types. Attention is directed to the relationship of these strains to *Escherichia paragruenthali* and *Escherichia acidilactici*, and the opinion is expressed that they can conviently be considered as chromogenic varieties of these recognized species, thus avoiding the necessity for the creation of new species.

REFERENCES

BERGEY, D. H. 1934 Manual of Determinative Bacteriology. 4th Edition, 664 pp. Baltimore, Md. The Williams & Wilkins Company.

LEHMANN, K. B., NEUMANN, R. O., AND BREED, R. S. 1931 Bacteriology, Especially Determinative Bacteriology. Volume II. 7th Edition, 868 pp. New York. G. E. Stechert and Company.

PARR, L. W. 1937 Unrecorded form of *Bacterium aurescens*, sole colon-group representative in a fecal specimen. Proc. Soc. Exper. Biol. and Med., **35**, 563–565.

PAULSON, M. AND BROWN, J. H. 1932 The appearances produced in blood agar by bacteria of the *Bacterium coli* group. Jour. Inf. Dis., **51**, 407–411.

RIDGWAY, R. 1912 Color Standards and Nomenclature. Baltimore, Md. A. Hoen and Company.

TITTSLER, R. P. 1937 Chromogenic strains of *Escherichia* cultures. Jour. Bact., **33**, 450.

TITTSLER, R. P. AND SANDHOLZER, L. A. 1935 Studies on the *Escherichia-Aerobacter* intermediates. I. Cultural characteristics. Jour. Bact., **29**, 349–361.

STUDIES OF FRESHWATER BACTERIA

V. The Distribution of Siderocapsa treubii in Some Lakes and Streams[1]

YVETTE HARDMAN and ARTHUR T. HENRICI

Department of Bacteriology, University of Minnesota, Minneapolis, Minnesota, and the Wisconsin Geological and Natural History Survey, Madison, Wisconsin

Received for publication August 1, 1938

The genus *Siderocapsa* was created by Molisch in 1909 to include certain bacteria of striking morphology, found in fresh waters. Two species were described, *Siderocapsa treubii* (Molisch 1909) and *Siderocapsa major* (Molisch 1910). The former was found growing as a periphytic organism upon the leaves of submerged plants. It was described as coccoid, from 0.4μ to 0.6μ, occurring in colonies of not more than 8 cells, included in colorless, apparently mucoid material, the latter surrounded by a heavy irregular deposit of iron. Molisch described the cells as unstainable with the usual dyes, but staining red with Schiff's reagent for aldehydes. *Siderocapsa major* was found in the thin scum floating on the surface of stagnant pools. It differed from the preceding species in the somewhat elongated form of the cells, up to 1.8μ in length; the mucoid material was not so sharply delineated from the iron, and the colonies were considerably larger, up to 100 or more cells.

Molisch's observations apparently remained unconfirmed for some years. Cholodny (1926) doubted the existence of these forms as iron-depositing bacteria. He pointed out that ferric hydroxide is deposited upon the leaves of submerged plants independent of any bacterial activity, and also appears in the water scum, and that bacteria are found in the same places. He

[1] Aided by grants from the Graduate School, University of Minnesota, and from the Wisconsin Alumni Research Foundation.

looked upon the organisms described by Molisch as an accidental association of various bacteria with independently deposited iron.

Naumann (1928), however, observed the growth of these bacteria upon glass slides submerged in various waters, and confirmed Molisch's description. Naumann also observed another species, *Siderocapsa monoica*, occurring as single cells surrounded by an iron deposit. This species was found only once, growing upon the leaves of *Potamogeton natans*. On the basis of this single observation he divided the genus into two sections: *Monosiderocapsa* to include *S. monoica*, and *Polysiderocapsa* to include the two species named by Molisch.

A fourth species, *S. coronata*, was described by Redinger (1931). This is a plankton organism, i.e., it floats free in the water unattached to any surface. He points out that this habit of growth invalidates Cholodny's argument, since no plant material other than the bacteria is involved. It was found in Upper Lake Lunz, in Austria, and appeared in large foamy masses, at times as large as a fist. These zoogloeae were composed of coccoid bacteria, about 1μ in diameter, occurring in groups of 2 to 8 cells surrounded by mucoid material upon which iron and manganese were deposited in large quantities. Ruttner (1937) reported further on the distribution of this organism, which was found to be related to the oxygen stratification in various Alpine lakes. It was found most frequently at depths from 17 to 20 meters, where the oxygen range was from 0.12 to 0.30 mgm. per liter. The highest oxygen tension at which it was found was 4.66 mgm. per liter.

Dorff (1934), working with Naumann, reobserved *S. treubii* and *S. major*. The former species was found to stain in varying degrees with basic aniline dyes, although warming the stain was necessary in some cases. *S. major* was found not only in the scum on stagnant pools, but also upon the leaves of submerged plants. Both species were found only in still waters.

We have observed an organism presenting the characters of the genus *Siderocapsa* as described by Molisch, on slides which have been suspended in the waters of various lakes and streams. In many instances the iron deposit surrounding the colonies of

this organisms was the only iron deposit upon the slides. We believe, therefore, that this iron is deposited by the activities of the bacteria in question, and is not a mere accidental occurrence as proposed by Cholodny. We have found, contrary to the observation of Dorff, that these bacteria may grow abundantly on slides submerged in very rapidly flowing streams.

We have found upon our slides types of colonies which correspond in part with the two species described by Molisch. The first type has coccoid cells, 0.6μ to 1.6μ in diameter. From one to 30 cells are found in a colony, most frequently 6 to 8. The cells tend to occur in pairs, and stain deeply with Hucker's ammonium-oxalate crystal-violet solution. The outer edge of the mucoid capsule is well defined; it is round to elliptical, and may be from 2μ to 8μ across, most often about 4μ. Several colonies may be surrounded by a single continuous deposit of iron. This organism fits, in most particulars, Molisch's description of *S. treubii*.

The second type, far more common, is a small rod, 0.4 to 1.0μ in diameter and 2μ in length. The cells often stain unevenly. The colonies are usually elliptical in form, the mucoid capsule averaging about 2μ by 4.5μ. From 2 to 30 cells may be found in a colony, usually 4 to 6. Although the cells are rod-shaped, the other characteristics of this organism fit Molisch's description of *S. treubii* as well as those of *S. major*.

In all colonies the outer deposit surrounding the mucoid capsule, whether light or heavy, is rust brown when unstained, and is probably composed entirely of iron oxide. When the slides are stained with potassium ferrocyanide in dilute hydrochloric acid (the Berlin blue test for ferric iron), and counterstained with erythrosin, the cells are pink, the capsular material clear, and the whole outer structure blue. An attempt to stain one slide with Schiff's reagent failed. The slide was, however, several years old.

Although we have found on our slides forms corresponding fairly well with both *S. treubii* and *S. major*, we have also found, even on the same slide, so many intergrading forms, that we seriously question whether a differentiation of these two species

can be justified. It was observed that, when the colonies occurred in clusters, the individual colonies within a cluster were all of a single type as regards size and form of the cells. But the finding of so many doubtful intermediary forms prevented any recording of the two "species" separately. We shall refer to the organisms we have observed as *Siderocapsa treubii*, with the understanding that by this name we mean both types described by Molisch as well as the numerous intermediate forms.

The morphology of this organism as it occurs in its natural habitat is so striking that it can readily be recognized. The iron-encrusted colonies may be readily found with the low-power lens of the microscope. It is therefore an ideal organism for an ecological study by the direct microscopic method. We have searched for *Siderocapsa treubii* on nearly 2000 slides which have been suspended in the waters of 12 lakes and two streams in Minnesota and Wisconsin. These waters have varied widely in their physical and chemical characteristics, and it has therefore been possible to determine something about the environmental factors which determine the distribution of the microbe.

Although there was obviously a variation in the number of colonies on different slides no attempt has been made to count them. The organism has merely been recorded as "present" or "absent." In all instances several observations have been made at different times. All observations have been made during a period of the year when there was no ice in the lakes, with the exception of Lake Mendota, where observations were also made during the winter. In each lake, slides were submerged near the deepest part of the lake, being suspended at intervals from top to bottom. In the case of Lake Mendota and Lake Alexander, slides were also placed at several stations near the shore.

The occurrence of *Siderocapsa* in the various lakes and streams together with certain physical and chemical data regarding the lakes are presented in table 1. The chemical data have been obtained from various publications of Birge and Juday and their associates from the Wisconsin Geological and Natural History Survey. The chemical analyses recorded have been made on

surface samples. In those lakes where *Siderocapsa* is recorded as present, it was found at all depths, excepting the two deep, thermally-stratified lakes, Trout and Mendota.

In Trout Lake the organism was found in increasing abundance from the surface to a depth of 8 meters, below which level it was completely absent. The thermocline in this lake is found at about 12 meters, at which level the dissolved oxygen, tempera-

TABLE 1

Occurrence of Siderocapsa treubii in different types of lakes

LAKE	TYPE	OCCURRENCE OF SIDEROCAPSA	pH	BOUND CO_2	TOTAL Fe	ORGANIC C
				mgm. per liter	*mgm. per liter*	*mgm. per liter*
Forestry Bog	Seepage	Absent	5.2	1.0	0.32	12.3
Helmet	Seepage	Absent	5.7	2.0	0.59	25.3
Weber	Seepage	Absent	6.0	1.5	0.03	2.8
Crystal	Seepage	Absent	6.0	1.5	0.07	1.6
Mary	Seepage	Absent	6.0	2.5	0.06	17.4
Muskellunge	Seepage	Absent	7.0	9.5	0.03	6.4
Boulder	Drainage	Present	7.5	13.6	0.48	7.7
Manitowish River*		Present				
Trout	Drainage	Present	7.5	19.0	0.03	3.7
Brazell	Drainage	Present	8.0	14.0	1.70	25.7
Little John	Drainage	Present	8.4	16.0	0.23	4.9
Allequash Creek†		Present	8.6	16.6	0.32	6.7
Mendota	Drainage	Present	8.7	34 to 70	0.20	7.1
Alexander	Drainage	Present	Alkaline	Hard		

* Manitowish River empties into Boulder Lake; presumably the water is similar in composition.

† Allequash Creek drains Allequash Lake. Chemical analyses have been made upon the surface water of the lake.

ture and pH all decline rapidly. In Lake Mendota *Siderocapsa* was found only rarely, and its distribution was quite erratic. It was found once at a depth of 9 meters in August, but did not occur on any other slides submerged during the summer, either in deep water or near the shore. During the winter and spring, however, it was found abundantly at a depth of 18 meters, i.e., 2 meters above the bottom, and absent at all higher levels, save for single recordings at 15 meters and 12 meters.

Lake Alexander has been referred to in previous papers of this series as a seepage lake, and this is true at the present time. But until about ten years ago it drained into the Mississippi, and it presents the characteristics of a drainage lake. While, unfortunately, no chemical analyses are available, its water is obviously very hard and alkaline. *Siderocapsa* has been found on nearly all of the many slides which have been submerged in this lake, but is clearly much more abundant in the shallow weed-choked bays than in the open lake. While it has been found at all depths, it is more abundant on slides about 1 meter above the bottom than at other levels. Lake Alexander is not stratified.

The slides placed in the Manitowish River and Allequash Creek were stationed at places where the current flow was very rapid. In Allequash Creek slides were placed in a rapid where the current flow was so great that some of them were torn loose from their attachment. Nevertheless, these slides developed a heavy coating of bacteria in a few days, including abundant specimens of *Siderocapsa*.

The data presented in table 1 show clearly that *Siderocapsa treubii* occurs characteristically in alkaline, hard water lakes of the drainage type, and is absent in neutral or acid, soft water lakes of the seepage type. The distribution is clearly not determined either by the organic matter or by the abundance of iron in the water, although possibly on a quantitative basis correlations with these characters could be established. The amount of iron cannot, however, be the sole determining factor. Slides from Forestry Bog and Helmet Lake, with high iron contents, showed an abundance of other types of iron-depositing bacteria, especially *Leptothrix crassa* and related forms, but *Siderocapsa* was completely absent. Conversely, the latter microbe was fairly abundant at the surface of Trout Lake, with a very low iron content (0.03 mgm. per liter), and absent at the bottom, where there was much more iron (0.35 mgm. per liter) but a lower pH.

Of the various environmental factors considered, the pH of the water appears to be most important. Since *Siderocapsa treubii* is found only in alkaline waters, it seems highly probable

that it deposits iron by the oxidation of organic compounds, so-called "humates" of iron, the reaction probably occurring at the surface of the capsule. It has been shown by Halvorson and Starkey (1927) that truly autotrophic iron bacteria, i.e., those obtaining energy from the oxidation of inorganic ferrous to ferric compounds, could grow only in waters acid in reaction, since only in such waters could ferrous compounds remain in solution; and Turowska (1930) has shown that such organisms as *Leptothrix ochracea* and *Gallionella ferruginea* are rather sharply limited to waters neutral or acid in reaction. It is possible that the iron oxide is precipitated in the water spontaneously and merely adsorbed onto the capsule of *Siderocapsa*. This appears to be improbable because, as was mentioned, in many cases the only iron found on the slides was that deposited about the colonies of this microbe, although other types of bacteria or algae with mucoid capsules were frequently found. We therefore are of the opinion that *Siderocapsa treubii* is a heterotrophic bacterium which utilizes the organic radicle of organic iron compounds, depositing the iron as a waste product.

SUMMARY

Bacteria presenting the morphological characters of *Siderocapsa treubii* Molisch and *Siderocapsa major* Molisch were found upon glass slides submerged in various lakes and two streams. So many intermediate forms were encountered that it was considered impossible to separate these two species.

Siderocapsa treubii was present in all of the alkaline, hard water lakes of the drainage type, and absent in all of the neutral or acid, soft water lakes of the seepage type. It was found abundantly in two rapidly flowing streams.

Siderocapsa treubii is probably a heterotrophic organism utilizing the organic radicle of organic iron compounds and depositing the iron as a waste product upon the capsules of the colonies.

REFERENCES

Birge, E. A. and Juday, Chancey. 1934 Particulate and Dissolved Organic Matter in Lakes. Ecology Monographs, **4**, 440–474.

Cholodny, N. 1926 Die Eisenbakterien. Pflanzenforschung, Heft **4**, 1–162.

DORFF, P. 1934 Die Eisenorganismen. Pflanzenforschung, Heft. **16,** 1–62.

HALVORSON, H. O. AND STARKEY, R. L. 1927 Studies on the transformations of iron in nature. I. Theoretical considerations. Jour. Phys. Chem., **31,** 626–631.

JUDAY, C. AND BIRGE, E. A. 1932 Dissolved oxygen and oxygen consumed in lake waters of northeastern Wisconsin. Trans. Wis. Acad. Sci. Arts and Lett., **27,** 415–486.

JUDAY, C. AND BIRGE, E. A. 1933 The transparency, color and specific conductance of the lake waters of northeastern Wisconsin. Trans. Wis. Acad. Sci. Arts and Lett., **28,** 205–259.

JUDAY, C., BIRGE, E. A. AND MELOCHE, V. W. 1935 The carbon dioxide and hydrogen ion concentration of the lake waters of northeastern Wisconsin. Trans. Wis. Acad. Sci. Arts and Lett., **29,** 1–82.

MOLISCH, H. 1909 *Siderocapsa treubii* Molisch, eine neue, weit verbreitete Eisenbakterie. Ann. du Jard. Buitenzorg, 3me. Suppl. **29.** Quoted from Cholodny.

MOLISCH, H. 1910 Die Eisenbakterien. Quoted from Cholodny.

NAUMANN, E. 1928 Siderogone Organismen und die Bildung von Seenerz. Ber. d. Dtsch. Bot. Ges., **46,** 141. Quoted from Dorff.

REDINGER, K. 1931 *Siderocapsa coronata* Redinger, eine neue Eisenbakterie aus dem Lunzer Obersee. Arch. f. Hydrobiol. **22,** 410–414.

RUTTNER, F. 1937 Limnologische Studien an einigen Seen der Ostalpen. Arch. f. Hydrobiol., **32,** 167–319.

TUROWSKA, I. 1929 Études sur les conditions vitales des bactéries ferrugineuses. Bull d. l'Acad. Polonaise des Sci. et des Lett. Classe des Sci. Math. et Nat., Series B: Sci. Nat. (1), 255–282.

PLATE 1

FIG. 1. A colony of *Siderocapsa treubii* with numerous coccoid cells.

FIG. 2. A colony with paired coccoid cells.

FIG. 3. A colony with rod-shaped cells.

FIGS. 4 and 5. Confluent or multiple colonies.

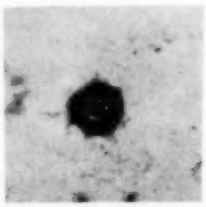


Fig.1

Fig.2

Fig.3

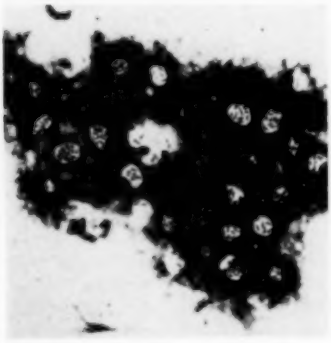


Fig.4

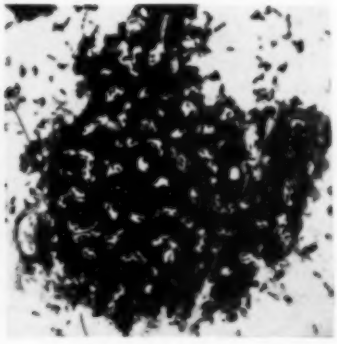


Fig. 5

PROCEEDINGS OF LOCAL BRANCHES OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

EASTERN PENNSYLVANIA CHAPTER

FIRST MEETING OF THE CURRENT SEASON, PHILADELPHIA COUNTY MEDICAL SOCIETY BUILDING, OCTOBER 18, 1938, PHILADELPHIA, PENNA.

THE ORIGINAL MINUTES OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS. *C. G. Roos*, Sharp and Dohme Laboratories, Glenolden, Penna.

Announcement was made of the finding of the original minutes of the first meetings of the Society of American Bacteriologists. While it was known that some records had been left at Glenolden in 1917 by the Society's secretary, Dr. A. Parker Hitchens, a post-war search had been fruitless. In the spring of 1938 the laboratories in the main building were remodeled and provision made for safe keeping of valuable records. While old records of value were being gathered, the record book containing the minutes of the Society's first meetings was brought to light.

The original minutes are contained in a small record book consisting of 200 pages. The 101 inscribed pages include the original transcript of the Society's constitution, minutes of the meeting of organization, names of the Society's organizers with a list of the names of its charter members, the first to the eleventh programs presented at the annual meetings and the minutes of transactions at those meetings. The earliest date recorded is October 16, 1899 and the last December 30, 1909.

Photostats are being made of the records and will be available at the library of the Mulford Biological Laboratories of Sharp and Dohme, Glenolden, Pa. The original record book will be delivered to the Archives Committee of the Society of American Bacteriologists.

THE PATHOGENESIS OF LOCAL TETANUS. *William Chalian*, Laboratory for Endocrine Research, The Johns Hopkins University, School of Medicine, Baltimore, Md.

Theories of the pathogenesis of tetanus were reviewed. The nerve-blocking experiments of Meyer and the dynamic-activation experiments of Doerr and Seidenberg were shown to be defective and hence of no value to the axis-cylinder-carriage theory. The fact that denervated muscles fail to respond to the injection of tetanus toxin does not preclude the possibility of a peripheral action of this poison. The following theory is upheld: Tetanus toxin can be carried to the tissues of the body, whether these be specifically reacting striated muscles or motor horn cells or structures that do not respond in a recognizable manner to the action of the toxin, *only by the same mechanisms that effect the distribution of countless other drugs and poisons—the blood and the lymph-vascular systems.* The toxin is capable of exhibiting a dual action, the components of which are separately demon-

strable. The first effect, which is a poisoning of the motor horn cells, results in the appearance of convulsions and reflex spasms. The second effect is exerted directly upon the motor end-organs present in striated muscle and is manifested by the development of muscular rigidity.

A detailed report of this work will appear shortly in the Bulletin of the Johns Hopkins Hospital.

RECENT ADVANCES IN OUR KNOWLEDGE OF TETANUS. *Warfield M. Firor*, Johns Hopkins University, School of Medicine, Baltimore, Md.

Long ago we became interested, with Dr. Abel, in the study of tetanus. Dr. Abel subjected the neural transport theory of Meyer to a critical review and concluded that tetanus toxin is absorbed by the lymphatics and distributed by the blood stream. He felt that the rigidity seen in local tetanus is due to a peripheral action of the toxin on the neuromuscular end-organs and that the hyperexcitable reflexes are central nervous system manifestations. To prove this latter point we injected into the gray matter of the lumbar cord of dogs minute amounts of tetanus toxin. With as little as 1/300th ordinary lethal dose we were able to produce tactile reflex tetanus. All these animals died. Further studies showed that following intraspinal injections, death results, regardless of whether the afferent and efferent nerves are severed or the spinal cord divided above the point of injection. These observations led us to hypothecate that tetanus toxin is altered to form a new substance. Experiments show that intravenous administration of tetanus antitoxin does not prevent death following the intraspinal injection of tetanus toxin. Experiments in which an injected animal is cross-circulated with a normal one seems to bear out the assumption that tetanus toxin is altered within the spinal cord to form a new substance.

ONE HUNDRED AND THIRTY-FOURTH MEETING, PHILADELPHIA COUNTY MEDICAL SOCIETY BUILDING, PHILADELPHIA, PA., NOVEMBER 22, 1938

A SIMPLIFIED COMPLEMENT FIXATION TECHNIC FOR THE SEROLOGICAL DIAGNOSIS OF SYPHILIS. *Fred Boerner and Marguerite Lukens*, Graduate Hospital, University of Pennsylvania, Philadelphia, Pa.

A simplified technic for a qualitative or quantitative complement-fixation test is described which effects marked reduction in cost and labor. Chances of error are minimized by a reduction in the number of steps and by the fact that omission of any step is readily detected.

For the qualitative test, one dose of serum is employed; additional doses are used for the quantitative test.

Qualitative test: (1) Add 0.1 cc. of each serum to be tested to each of 2 tubes. Inactivate in water-bath, 58°C. for 10 min. Also include suitable controls. (2) To tube 1 add 0.5 cc. antigen-complement mixture (antigen diluted in 1:30 complement so 0.5 cc. contains proper dose of antigen). (3) To tube 2, add 0.5 cc. complement 1:30. (4) Mix tubes thoroughly, place in refrigerator (6°–10°C.), 15–18 hours. (5) Place in water-bath, 37°C., 10 minutes. (6) To both tubes add 0.5 cc. 0.75% sensitized corpuscles. (The corpuscles and hemolysin should be mixed at least 15 minutes before use.) (7) Mix thoroughly, place in water-bath, 37°C., for 1 hour. (8) Reactions are interpreted as follows: ++++,

positive; +++, ++, + or ±, doubtful; −, negative. (9) When several doses of serum are used for the purpose of making a quantitative determination of the degree of positiveness of the serum, the reactions obtained with each dose are recorded for comparison with previous or subsequent tests.

A Simplified Method for the Preparation of an Antigen for Use in Complement Fixation Test for Syphilis. *Fred Boerner, Charles A. Jones and Marguerite Lukens*, Graduate Hospital, University of Pennsylvania, Philadelphia, Pa.

A simplified method for preparation of antigen for use in the above complement-fixation test for syphilis is described. (1) Place 10 gms. powdered beef heart (Difco) in 1 or 2 l. flask. (2) Add 225 cc. absolute alcohol and 75 cc. ether (U. S. P.); allow to stand at room T. ½ hour, shaking thoroughly every 5 minutes. (3) Filter through filter paper, discard the residue. (4) Place the filtrate in large flask and evaporate, using slight suction, to 50 cc. by boiling in a water-bath. If the concentrated extract is less than 50 cc., add sufficient absolute alcohol to make up to volume. (5) Place the concentrated filtrate in the refrigerator for 1 hour. A heavy precipitate will form. (6) Filter through paper in the refrigerator so that the filtration will be completed while the solution is cold. (7) Allow the filtrate to stand at room T. overnight; if a precipitate forms, filter. (8) Dissolve 200 mgms. cholesterol in the 50 cc. of filtrate. This finished antigen is now ready to be tested for its optimum fixing dose and anti-complementary properties.

If desired that the antigen be more or less sensitive, merely vary the cholesterol content. Antigens prepared by the above method are slightly more anti-complementary than the alcohol used in their preparation, and their optimum antigenic dose is usually between 1:800 and 1:1,600. Any volume of antigen can be prepared by this method by proportionally increasing or decreasing the amounts.

Report of the Conference on the Laboratory Diagnosis of Syphilis at Hot Springs, Ark., and the Meeting of the Laboratory Section of the American Public Health Association in Kansas City, Mo. *A. Parker Hitchens*, University of Pennsylvania, School of Medicine, Philadelphia, Pa.

WASHINGTON BRANCH

Army Medical School, Washington, D. C., November 15, 1938

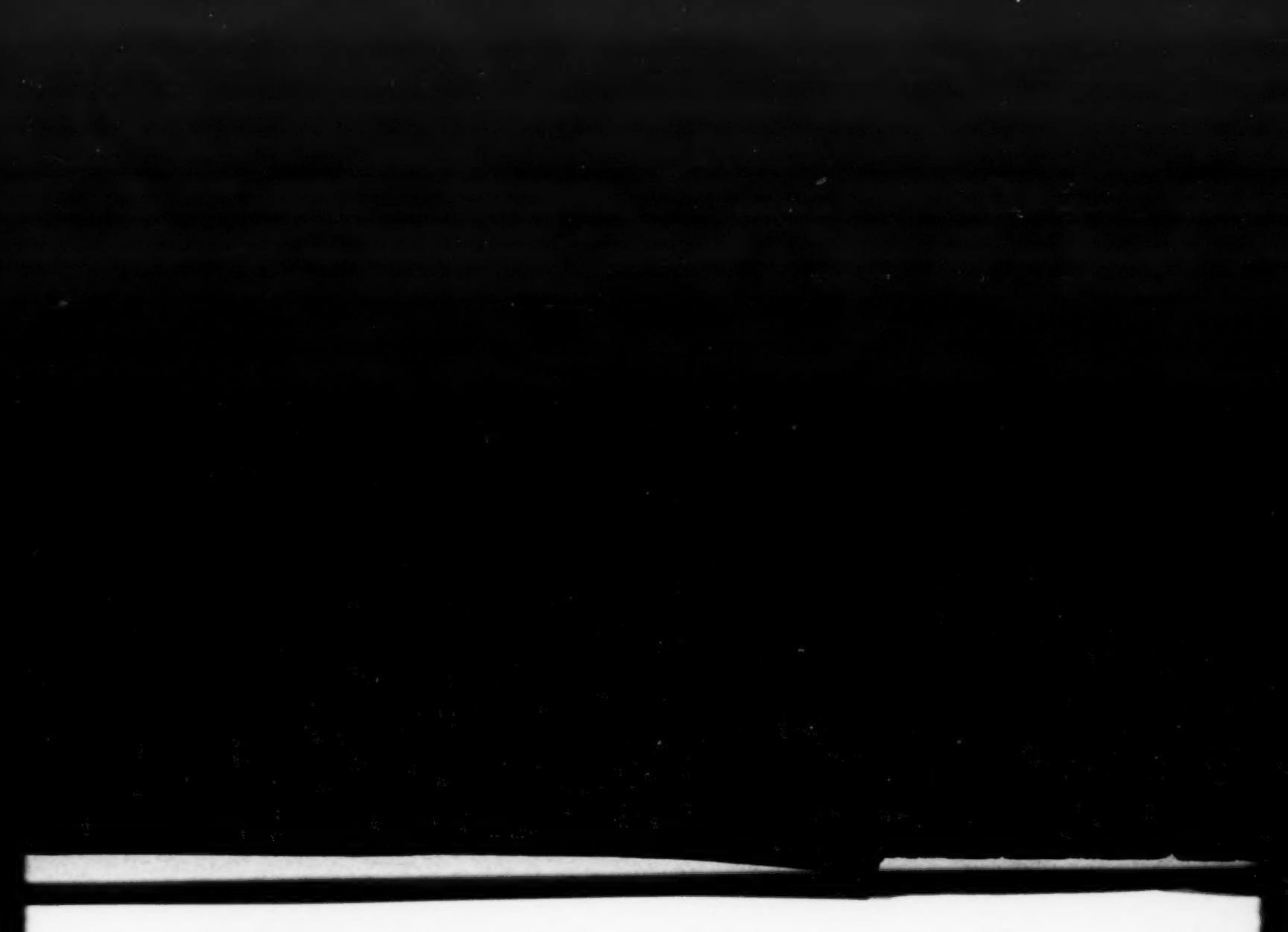
Mechanism of the Action of Sulfanilamide in Brucellosis. *Henry Welch*, Food and Drug Administration, U. S. Department of Agriculture.

In cases of human brucellosis and in guinea pigs infected with *Brucella abortus*, treatment with sulfanilamide markedly increases phagocytosis of this organism. Individuals infected with other diseases and normal guinea pigs treated with sulfanilamide show no change in their opsonocytophagic activity for *Brucella abortus*. Further, the blood of normal human beings or normal guinea pigs, when treated *in vitro* with sulfanilamide, shows no change in opsonocytophagic activity

when mixed with untreated *Brucella abortus* or with sulfanilamide-treated organisms.

It has been possible in infected guinea pigs, treated with sulfanilamide, to demonstrate the absence of the drug in the blood at the time at which marked phagocytosis occurs, and, further, to transfer this marked phagocytic activity to normal guinea pig and human cells by suspending them in the serum of infected treated guinea pigs. Since the presence of sulfanilamide as such is not essential for the demonstration of marked phagocytosis in infected treated guinea pigs, it would appear that the effect of the drug is an indirect one.

The marked phagocytic activity demonstrated with normal cells in the presence of sera from infected individuals treated with sulfanilamide is completely lost by the addition to such sera of small amounts of a concentrated filtrate of *Brucella abortus*.

It would appear that sulfanilamide acts indirectly on *Brucella* infections by increasing the opsonic power of the blood and thus neutralizing the effect of the endotoxin or aggressin-like substances produced by this organism allowing phagocytosis to take place. The fact that it was not possible to stimulate the opsonic power of the blood toward *Brucella* of uninfected human beings or guinea pigs by treatment with sulfanilamide would indicate that an infective or immunizing process must be in progress for the drug to stimulate phagocytosis in this disease.

PROBLEMS IN PNEUMONIA. *Lloyd Felton*, National Institute of Health.